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PRINCIPAL INVESTIGATOR: Thomas Inui

PI ADDRESS: University of Washington
Harborview Medical Center
325 Ninth Avenue
Seattle, Washington 98104

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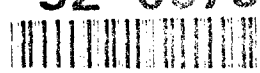

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236 persons (cases) with past reactive human immunodeficiency virus type-1 (HIV-1) EIAS and indeterminate HIV-1 Western Blots (IWBS) were followed prospectively. Of the 159 cases followed ≥ 6 months, 6 seroconverted (3.8% seroconverstion risk). All 6 seroconverters reported HIV risk behaviors, all had p24 bands prior to seroconversion, and 5 of the 6 seroconverted within one month.

HIV-1 culture, polymerase chain reaction, serum p24 antigen, and the recombinant assays, CBre 3 and Syva Microtrak, had specificities of 100%, 99.1%, 100%, 97.1%, and 100%, respectively. An algorithm for evaluation of persons with IWB is proposed.

A case - control study demonstrated past positive tuberculin skin test, recent tetanus booster, autoantibodies, and parity were associated with IWBs. No cross-reactivity was seen with HTLV-1 or HIV-2.

Cases reported significantly more anxiety than controls, due in large part to the IWB. High-risk cases reported more anxiety than low-risk cases. Informational brochures for counseling were designed.

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Thomas S. Jr. 9/24/91

PI Signature Date

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TABLE OF CONTENTS

INTRODUCTION

The first laboratory step in human immunodeficiency virus type-1 (HIV-1) antibody detection is the enzyme-linked immunosorbent assay (EIA) which has a reported sensitivity and specificity of greater than 99% [1-5]. Those specimens that are repeatedly reactive by HIV-1 EIA are confirmed by a more specific supplemental test, which is usually the Western blot. The Western blot detects antibodies to specific denatured HIV-1 proteins, such as the core proteins (p17, p24, and p55), polymerase proteins (p31, p51, p66), and envelope proteins (gp41, gp120, gp160) [6-8]. The Western blot has a reported specificity of 97.8% [5]. Approximately 4-20% of sera that are repeatedly reactive by HIV-1 EIA are interpreted as indeterminate by Western blot [8-11].

Indeterminate HIV-1 Western blots (IWB) may be due to antibody production against viral core antigens early in HIV-1 infection [12-14], loss of core antibodies late in HIV-1 infection [15,16], cross-reactive antibody to HIV-2 [17], or cross-reactive antibody due to autoantibodies or alloimmunization [18-21]. The etiologies of IWB have not been well-characterized; the reported associations of IWB with other medical conditions or cross-reactive antibodies generally are based on case series. However, Dock and colleagues compared blood donors with IWBs with seropositive and seronegative blood donor controls and found no difference in frequency of past cytomegalovirus, Epstein-Barr virus, hepatitis A and B virus, and herpes virus simplex virus 1 infections between the IWB and seronegative group [21].

Due to the possibility that an IWB represents recent HIV-1 infection and incomplete antibody production, the CDC recommends that all individuals with IWB be retested over six months. The CDC recommends that a low-risk individual be considered "HIV-negative" if the Western blot is still indeterminate or becomes negative after six months. Longer follow-up, HIV-1 testing of sexual and drug-using partners, and additional immunologic and virologic evaluation are recommended for high-risk individuals with IWB [8].

Individuals with IWB are currently excluded from blood donations and have had difficulty obtaining life and disability insurance, U.S. immigration status, and visas for

foreign travel. Concern about possible HIV-1 infection among individuals with IWB has resulted in uncertainty about appropriate procedures for notification, counseling, and evaluation. A clearer estimation of the risk of seroconversion among individuals with IWB is needed. Accurate identification of HIV-1 infection among individuals with IWB may be possible using supplemental HIV-1 tests, including HIV-1 culture, serum p24 antigen, polymerase chain reaction, radioimmunoprecipitation assay, and recombinant HIV-1 antigens, but the sensitivity and specificity of these supplemental tests in individuals with IWB is not known.

This study was designed to assess the risk of seroconversion and the specificity of supplemental HIV-1 tests in a prospective cohort of both low- and high-risk individuals referred because of repeatedly reactive EIAs and IWB. In addition, a case-control study was conducted to determine risks for IWB, comparing the cases who did not seroconvert with seronegative (HIV-1 EIA negative controls). Finally, the impact of IWBs was measured in terms of anxiety and depression comparing the cases and controls and comparing high- and low-risk cases with regards to their concerns about the IWB.

METHODS

Study Population

A prospective cohort study with six to nine months follow-up was initiated at the University of Washington in March 1988. The cohort included men and women 16-70 years of age with one or more repeatedly reactive EIA and IWB in the past, who were referred from testing sites in Washington and Oregon states. We accepted the HIV-1 Western blot interpretative criteria of the referral laboratory for Western blots performed on subjects prior to study enrollment. Individuals with a prior diagnosis of HIV seropositivity or AIDS were excluded from the study. After informed consent was obtained, cases were interviewed about HIV risks [22] and general medical history, and were examined. Cases were encouraged to refer their current sexual partner(s) to the study for evaluation and HIV-1 antibody testing.

Controls who had a negative HIV-1 EIA within the past three months were recruited from the same HIV-1 testing sites as the subjects and were frequency-matched by HIV-1 testing site (eg., blood bank or sexually-transmitted disease clinic). After informed consent, controls were interviewed about HIV risks and general medical history, were examined and HIV-1 serologies were repeated. Controls were not followed prospectively.

Risk of seroconversion

The two reference laboratories performing EIAs and Western blots for the study (the University of Washington Virology laboratory and the Washington State Public Health laboratory) subscribe to the College of American Pathologists Proficiency panel for HIV-1 antibody testing. The Washington State Public Health laboratory performed HIV-1 serologies for the study from March 1988 to November 1989 and the University of Washington performed serologies from November 1989 to August 1991.

Cases were followed prospectively with repeat HIV-1 EIAs and Western blots every three months. A subset of blood donors with IWBs referred from Portland American Red Cross were enrolled in the study, but could not be prospectively followed due to logistic difficulties (distance from the study site at the University of Washington). Thirty-nine blood donors with an interval of six months or longer between their initial IWB as a blood donor

and the first study visit are included in the analyses on the risk of seroconversion and the specificity of supplemental tests.

Dupont (Biotech Research Laboratory Inc., Rockville, MD) and Genetic Systems (Seattle, Wa) EIAs and Epitope (Beaverton, Or) Western blots were performed on study subjects. Dupont Western blots were performed on a subset of sera from study subjects. The CDC interpretive criteria were used for both Epitope and Dupont Western blots; a Western blot was considered positive if antibodies were present to two of the following HIV-1 viral proteins--p24, gp41, and gp120/gp160 [8]. Western blots without any bands were considered negative and blots with bands not meeting the criteria for a positive blot were interpreted as indeterminate. The diagnosis of HIV-1 infection was based upon seroconversion (a positive EIA and Western blot) or upon isolation of HIV-1 in culture from peripheral blood mononuclear cells (PBMCs). Positive Western blots were repeated to rule out laboratory error.

Specificity of supplementary HIV-1 tests

Determination of the specificity of supplemental tests was based on test results from the individuals who did not develop a positive HIV-1 culture or positive Western blot during six months or longer follow-up. Supplemental tests were performed on sera and cells obtained from cases at the first study visit and on samples from HIV-1 EIA negative controls recruited from the same HIV testing sites.

HIV-1 cultures and serum p24 antigen

Cell-free plasma and PBMCs were cultured for HIV-1 as previously described [23,24]. Culture supernatants were sampled for HIV-1 p24 antigen with the antigen capture EIA following the manufacturer's protocol (Abbott Laboratory, Chicago, Il) every three days for one month. Serum p24 antigen assays were performed, using the same antigen capture EIA method [25-27]. Positive serum samples were tested in a confirmatory antibody neutralization assay.

Polymerase chain reaction (PCR)

PCR was performed by CETUS Corporation (Emeryville, CA), Roche Biomedical Laboratories (Research Triangle Park, NC), and the University of Washgton Retroviral

laboratory using the SK38/39 and SK101/145 primer pairs for the HIV-1 gag gene [28,29]. SRA Technologies, Inc, in conjunction with Walter Reed Army Institute of Research Retroviral laboratory, also performed PCR for HIV-1 using the SK38/39 primers with confirmation of positive results with nef/LTR primers. SRA performed PCR for HIV-2 for two cases with residence in West Africa, using primer pairs SK 100/104 and the HIV-2 specific primer SK 89/90.

Cell lysates were obtained from cryopreserved PBMCs and amplification competency of specimens was checked by amplification of a conserved region within the HLA-DQ alpha locus with primer pair GH26/27 [30]. HIV-1 DNA amplification was performed as described by Kellogg and Kwok [28]. Each specimen was run in duplicate for both primer sets. HIV-1 proviral sequences were considered present if both primer pairs were positive in duplicate, indeterminate if only one of the duplicate reactions was positive for one or both primer pairs, and not present if neither primer pair resulted in a positive signal.

Serologic assays

Four serologic EIAs to recombinant HIV-1 antigens were performed: HIVAGEN® (SmithKline Beecham Clinical Laboratories, Van Nuys, CA), ENV 9® (Dupont Glasgow Research laboratory, Glasgow, DE), CBre3® (Cambridge Biosciences Laboratory, Cambridge, MA), and Microtrak ® (SYVA Corporation, Palo Alto, CA). The HIVAGEN panel was comprised of five recombinant HIV-1 antigens produced in Escherichia coli; Ip24 represents the entire sequence of p24, Kp55 the complete sequence of p55, Kp66/31 the complete reverse transcriptase genome and 40% of endonuclease, Kp41 40% of the N-terminus of gp41, and Igp120 98% of gp120 [31]. A HIVAGEN result that showed Ip24, Kp55, or Kp66/31 and either Kp41 or Igp120 was considered positive and any other pattern of reactivity was considered indeterminate. ENV 9 utilized a single HIV-1 envelope peptide (the carboxy terminus of gp120 and half of the gp41 sequence), produced in E.coli [32]. CBre3 is a FDA-approved recombinant-based ELISA that has proteins from two conserved gene products--the carboxy-terminal half of gp120, the amino-terminal half of gp41, and all of p24 with small portions of

p17 and p15 [33]. The SYVA Microtrak is manufactured from the same gene products as the CBre3.

Radioimmunoprecipitation assays were performed by Biotech laboratory (Biotech Research Laboratory, Rockville, MD) using HIV-1 infected H-9 cells, labelled for eight hours with S^{35} methionine (34). Samples reactive with the envelope glycoproteins, gp120 and gp160, were considered positive.

Risk factors for IWB

A case-control study was conducted comparing the cases who did not seroconvert with EIA negative controls to ascertain etiolgies for IWB other than acute HIV-1 seroconversion. Cases and controls were administered a questionnaire about medical history, including autoimmune diseases, viral diseases, parity, risk for HIV and other sexually-transmitted diseases (Appendix A). Due to the heterogeneity of cases referred from low-risk (i.e., blood banks) as well as high-risk testing sites (i.e., AIDS Prevention Project), cases and controls were frequency-matched by HIV testing sites and stratified by testing site in the analyses.

To assess risk factors for IWB other than HIV-1 infection, the nonseroconverter cases and controls were compared in terms of reported autoimmune illness, recent viral illness, past history of tuberculosis or a positive skin test for TB (purified protein derivative or PPD), parity, immunization and transfusion history, past STDs, and risk behaviors for HIV since 1978. Variables with a $p < 0.1$ on univariate analysis were potentially entered into stepwise multivariate runs using conditional logistic regression with entry criteria of $P < 0.05$.

The cases and controls were stratified into three groups (blood donors, high-risk testing sites of the AIDS Prevention Project and the STD clinic, and the women's and prenatal clinics) to try to reduce potential confounding variables introduced from the heterogenous mix of cases from different testing sites. Low-risk cases for whom random controls could not be enrolled (i.e., life insurance applicants and patients referred by private physicians) were matched to blood donor controls. The analyses were performed in several ways: i) for all nonseroconverter cases, ii) restricted to "incident" cases with initial IWB performed within six months prior to study enrollment, and iii) gender-specific analyses.

A screen for autoantibodies included antinuclear antibodies (ANA) and rheumatoid factor. A screen for other infectious diseases included a VDRL and hepatitis B surface antigen and antibody. HLA antibodies and EIAs for HTLV-1 and HIV-2 were performed in a subset of cases.

Psychological impact

At the end of the first study visit, cases and controls were asked to fill out a self-administered questionnaire about life events in the past six months (using the standard 14-item life event score with the addition of two items--the decision to be tested for HIV and the results of the HIV test), the 13-item Beck Depression Inventory, and the Symptom Checklist 90 (SCL-90) anxiety subscale. The estimated degree of depression according to the BDI score is 0-4=none or minimal, 5-7=mild, 8-15=moderate, and 16+=severe [35-37]. The SCL-90 anxiety scale was standardized to nonpsychiatric outpatients ; the minimum score is 36 for males and 38 for females [38]. The cases also were administered a nine-item questionnaire about their interpretation of and concerns about the IWB (Appendix B).

Statistical methods

Demographic and HIV risk factors were compared using Chi-square analysis and Fisher's exact test for categorical data and Student's t-test for continuous data. The Mann-Whitney test was used for comparing continuous distributions when the assumption of a normal distribution was not appropriate. Logistic regression was used to compare the proportion of cases with reactive versus nonreactive HIV-1 EIA at visit one with respect to the proportion with past high-risk sexual partners while controlling for time between initial HIV tests and study enrollment, and for the number of HIV tests prior to study enrollment. Specificity of the supplementary HIV-1 tests was analyzed by comparing results of the supplementary tests to the six month Western blot result and isolation of HIV-1 by culture as the gold standards. Ninety-five percent confidence intervals for the seroconversion risk were calculated using exact binomial methods.

Conditional logistic regression was used to compare the cases and controls for risks of indeterminate Western blots, stratified by three groups of testing sites--blood donors, high risk testing sites (AIDS Prevention Project and Sexually-transmitted Disease clinic), and women's and prenatal clinics. These analyses were limited to the cases who did not seroconvert within the study follow-up to ascertain the etiologies of IWB among the nonseroconverters. Analyses were performed for males and females combined and separately.

Additional univariate and multivariate analyses were performed, restricted to the cases who first tested indeterminate by Western blot within six months or less prior to study enrollment, termed "incident" cases for the purposes of the analyses. The purpose of the restricted analyses was to reduce the potential for misclassification from inclusion of cases who tested indeterminate up to 55 months prior to study referral since the factors associated with their indeterminate status might no longer be present at the initial study visit. These cases were primarily blood donors who previously tested indeterminate by Western blot and who were retrospectively notified of their ineligibility to donate blood on that basis.

Conditional logistic regression was performed to compare the cases and controls by anxiety and depression scores, stratified by testing site. Subgroup analyses among the cases included high- and low-risk cases, seroconverters and nonseroconverters, and pregnant versus nongravid women were compared by self-reported anxiety, interpretations of the IWB, and past psychiatric history.

RESULTS

Of 236 persons with indeterminate HIV-1 Western blots referred and enrolled in the study as of August 1991, 159 were followed for six months or longer and were included in the analyses of risk of seroconversion and specificity of supplemental tests. The remaining cases are still being followed prospectively. Sixty-three percent of cases were referred from blood banks and 31% from the Seattle-King County Department of Public Health clinics, primarily the AIDS Prevention Project and the Sexually-transmitted Diseases clinic, and 5% from women's and prenatal clinics. One hundred four (44%) cases elective sought HIV-1 testing due to concern over possible exposure to HIV-1 and the remaining 132 (56%) were routinely screened for HIV-1 screening as blood donors, military recruits, or life insurance or immigration applicants.

As of August 1991, 144 EIA negative controls were recruited from the same HIV-1 testing sites as the cases. Ninety-one (63%) controls were recruited from blood banks, 47 (33%) from the AIDS Prevention Project and Sexually-transmitted Diseases clinic, and 5 (4%) from prenatal clinics.

Seventy-nine sexual partners of cases were referred by their partners (the case) and enrolled in the study. Of the 79 cases' sexual partners enrolled in the study, 57 (72%) of the index cases were blood donors, 18 (23%) were from high-risk testing sites, eg., AIDS Prevention Project and the Sexually-transmitted Disease clinic, and 4 (5%) were from women's and prenatal clinics.

The demographics of the cases, their current sexual partners, and controls were similar, except for marital status with a higher proportion of married cases than controls, more years of education among controls, and higher family income among cases and cases' sexual partners (Table 1). A higher proportion of the sexual partners were married, monogamous, and reported fewer STDs and lower risk for HIV than the cases or the controls.

Risk of seroconversion

Given the CDC recommendation for six months follow-up of persons with IWB, the risk of seroconversion was ascertained in cases with $\geq$ six months follow-up beyond their

initial IWB. The seroconversion risk was 6 of 159 (3.8%; 95% confidence interval, 1.4% - 8.0%). Seroconversion was only seen among individuals with p24 bands on their initial Epitope Western blots. The risk of seroconversion among individuals with p24 bands on initial Epitope Western blot was 6 of 45 (13%; 95% CI, 5.0% - 26.8%). The risk of seroconversion was 6 of 17 (35%) among high-risk individuals with p24 bands on Epitope blots and 0 of 28 among low-risk persons with p24 bands on Epitope blots ($P < 0.01$). The median EIA sample to cut-off ratio (R-value) for the seroconverters was 3.6 (range 3.3 - 8.5) at the first study visit.

Case 1 was a bisexual man with a history of prostitution and intravenous drug use prior to HIV-1 testing in 1988. He reported symptoms of an acute viral-like syndrome in the month between the IWB (p24 antibody only) and the positive Western blot. Case 2 was a woman with a history of autoimmune disease who had unprotected sexual exposure with an HIV seropositive bisexual partner. Following her initial IWB with a p24 band only, she seroconverted by Dupont blot two weeks later and by Epitope blot four weeks later. Case 3 was a homosexual man with a viral-like syndrome who had p24 and weak gp160 bands (interpreted by the referring laboratory as indeterminate by the FDA/Dupont criteria) and three months later had antibodies against all viral proteins on Western blot. Case 4 was a homosexual man with a mononucleosis-like syndrome with a borderline EIA (R-value 0.9) and a faint p24 and weak gp160 on initial Western blot who one week later had a reactive EIA (R-value 1.8), p24, gp41, and gp120, gp160 on Western blot. Case 5 was a homosexual man whose current sexual partner was recently diagnosed with AIDS; Case 5 seroconverted with a p18, p24, p66, gp120/160 one month after he had a p24 only on Western blot. Case 6 was a homosexual man with a p24 band and intermittent p66 band until he seroconverted in the tenth month of follow-up. He reported ongoing high-risk sexual behavior during the study period.

Estimation of the sensitivity of the supplemental HIV-1 tests is not reliable, given the low number of seroconverters. Case 1 had a positive ENV 9 assay and RIPA at the time the initial Western blot showed a p24 band only. Lymphocytes were not available from the initial

HIV test performed at an anonymous HIV testing site. When the Western blot became positive one month later at his first study visit, his HIV-1 PBMC culture, CBre3, SYVA Microtrak, and HIVAGEN were positive, but plasma culture, PCR, and serum p24 antigen were negative; repeat PCR was positive three months after seroconversion. Case 2 had a negative serum p24 antigen, HIV-1 PBMC and plasma cultures, and indeterminate HIVAGEN, but a positive PCR, CBre3, SYVA Microtrak, ENV 9, and RIPA at the initial study visit when the Dupont Western blot detected p17, p24, gp41, and gp120/160 antibodies (two weeks after the initial Western blot had p24 antibody only). Case 3 was positive on all supplemental tests (HIV-1 PBMC and plasma culture, serum p24 antigen, PCR, CBre3, ENV 9, and HIVAGEN) at the first study visit when the Western blot had antibodies against all viral proteins. Specimens were not available from his initial testing three months earlier when the Western blot detected antibodies to p24 and gp160.

Case 4 had a positive serum p24 antigen (>1,000 U/ml) at the time his EIA was borderline (R-value=0.9) with a faint p24 and gp120 and a weak gp160 on Western blot. One month later his p24 antigen was negative, PCR, SYVA, CBre3, plasma and cell culture were all positive, and his EIA was 4.0 and Western blot showed all bands. Case 5 was tested at an anonymous testing site so that serum was not available from his initial sample which showed a p24 band only on Western blot. One month later when his Western blot showed p24, gp41, and gp120/160 bands, CBre3, SYVA, PCR, and cell culture were positive, but plasma culture and p24 antigen were negative. Case 6 had a negative HIV-1 PBMC and plasma culture, PCR, ENV 9, and RIPA, and indeterminate HIVAGEN (Ip24, Kp55) at his initial study visit when the Western blot showed a p24 band only. He seroconverted after 10 months of follow-up, with a history of high-risk behavior intermittently during the 10 months, and refused repeat supplemental testing.

Western blot and supplemental test results among nonseroconverters

Cases with six months or greater follow-up were divided into three groups for analysis based on the HIV-1 EIA and Epitope Western blot results on samples obtained at the first study visit. Those in Group 1 were the six individuals who seroconverted, five of whom

seroconverted by the first visit and one who seroconverted 10 months after study enrollment. Group 2 was comprised of 71 cases who were still repeatedly reactive by HIV-1 EIA with a R-value ≥ 0.8 at the first study visit. Group 3 was comprised of 82 cases who were no longer reactive by HIV-1 EIA with a R-value < 0.8 at the first study visit.

The demographics of the three groups were similar except for gender, marital status, and family income (Table 2). Group 1 had the highest proportion of males and Group 3 contained the highest proportion of married cases and cases with family income above $20,000. Five (83%) of the six seroconverters in Group 1 were bisexual or homosexual men compared to approximately 90% of Groups 2 and 3 reporting their sexual preference as heterosexual ($P<0.001$). The proportion of cases reporting at least one high-risk sexual partner since 1978 was significantly higher for Groups 1 and 2 (100% and 32%, respectively) than for Group 3 cases (7%) ($P < .001$). The median time from initial IWB to study enrollment was shortest for Group 1 cases (1.0 month, range 0.5 - 3 months), intermediate for Group 2 (3.0 months, range 0.5 - 51 months) and longest for Group 3 cases (13 months, range 0.5 - 55 months) ($P < .01$ for difference between Groups 1 and 3; $P < .05$ for Group 1 compared to Group 2; and, $P = .003$ for difference between Groups 2 and 3). When the proportion of cases with a high-risk sexual partner since 1978 was compared between Groups 2 and 3 while adjusting for the time between initial IWB and study enrollment, and for the number of HIV-1 EIAs before study enrollment, the difference in high-risk sexual partners was statistically significant ($P = <.001$).

The median R-value of the HIV-1 EIA was 1.7 (range 0.9 - 5.6) among Group 2 cases at the first study visit compared to 0.2 (range 0.06 -0.7) among Group 3 cases. Fifty-four (76%) of Group 2 cases had an IWB at the first study visit compared to 30 (37%) of Group 3 cases ($P <0.001$, Table 4). The majority of both Group 2 and 3 cases had gag reactivity on blot, either p17 or p24. The kappa statistic for agreement in p24 bands on Epitope and Dupont blots was 0.419 ($P < 0.001$ by McNemar's test).

Excluding the 6 seroconverters and the 39 blood donors from Portland who could not be followed after the first study visit, 61 (54%) of the remaining 114 Group 2 and 3 cases

remained indeterminate by Western blot at follow-up visits, 26 (23%) had a negative blot initially and had indeterminate Epitope blots later, 14 (12%) were always negative by blot, 11 (10%) went from indeterminate to a negative blot at follow-up, and two (2%) had a false positive blot. The two false positive blots were seen in blood donors with no reported risk factors for HIV; one had had p24 bands only on prior blots and had p24 and gp120/160 on Epitope at one study visit with negative HIV-1 culture, PCR, and SYVA Microtrak and subsequent blots with a p24 band only. The other blood donor was erred with previous Dupont blots showing gp41 and gp120/gp160 bands. Her HIV-1 culture, PCR, SYVA Microtrak, and CBre3 were negative. She denied receiving HIV-1 vaccine. Her husband was evaluated and had a negative HIV-1 EIA and Epitope blot.

The specificity of supplemental tests performed at the initial study visit was estimated in the 153 nonseroconverter cases who had negative or IWB after six months or longer follow-up (Table 5). All 85 HIV-1 cultures performed were negative in the nonseroconverters. The polymerase chain reaction assay was negative in 22 of 22 EIA negative controls (data not shown) and 111 of 112 (99.1%) Group 2 and 3 cases who did not seroconvert. One high-risk individual was initially positive by PCR (CETUS laboratories) but negative on repeat PCR testing of the same specimen by two different laboratories. During an additional nine months of follow-up he remained negative for HIV-1 by Western blot, culture, and four serial PCR assays.

ENV 9 EIA was performed for 62 nonseroconverters, 4 of whom had borderline reactivity (R-values of 1.1 - 1.4). Specificity of ENV 9 was 93.5% in the cases and 100% in 39 EIA negative controls (data not shown).

Serum p24 antigen testing was performed in 77 nonseroconverters; one was borderline reactive but not neutralizable with anti-p24 antibody, resulting in a specificity of 100%.

HIV-1 radioimmunoprecipitation assay was performed in 56 nonseroconverters, of whom 44 were negative (78.6%) and 12 were indeterminate (21.4%). The specificity of RIPA was 78.6% if the indeterminate RIPA were considered 'false positives,' or 100% if the weakly reactive results were excluded.

Syva Microtrak was performed in 53 nonseroconverters, all of whom were negative by the Microtrak EIA for a specificity of 100%.

CBre3 was performed in 34 nonseroconverters , one (3%) of whom was positive (CBre3 R-value of 1.6) for a specificity of 97.1%. The one case with a false positive CBre3 was a low-risk person with a Genetic Systems HIV-1 EIA of 5.5 and persitent p24 on Epitope blot during six months of follow-up.

HIVAGEN was performed in 85 nonseroconverter cases and 63 EIA negative controls. Fifty-eight (68%) of the 85 nonseroconverter cases were indeterminate, one (1%) was positive, and 13 (21%) of the EIA negative controls were indeterminate by HIVAGEN ($P < .001$). Of the 58 cases with indeterminate HIVAGEN results, 36% and 70% had reactivity against the Ip24 and Kp55 antigens respectively, confirming the gag reactivity on Western blot. The specificity of the envelope antigens was 100% for Igp120 and was 98.8% for kp41 among the cases.

In summary, excluding indeterminate RIPA and HIVAGEN results, false positive PCR (N=1), CBre3 (N=1), ENV 9 (N=4), or HIVAGEN (N=1) results were obtained from seven cases, none of whom were positive on more than one supplemental test.

HIV serologies among controls and cases' sexual partners

The results of the EIA and Western blots among the controls and cases' sexual partners are displayed in Table 3. All 144 controls were EIA negative and 29 (23%) were indeterminate by Epitope blot. Five (6%) of the cases' sexual partners were repeatedly reactive by HIV-1 EIA and 17 (22%) were indeterminate by Epitope blot, compared to 24 (43%) of 56 of the sexual partners were indeterminate by Dupont blot. Three of the sexual partners were HIV positive; one was a bisexual man who did not know his serostatus and infected the one female seroconverter. The other two seropositive sexual partners included a hemophiliac whose wife (the case) did not serconvert and had negative HIV-1 culture, PCR, and recombinant ENV 9, and one was a married woman with few previous heterosexual partners and no other risks for HIV whose West African husband (the case) had a stable p17 band and was negative

for HIV-1 by culture and PCR and negative for HIV-2 by PCR. Supplemental tests for the sexual partners are still being analyzed.

Risk factors for IWB

The results of the univariate and multivariate odds ratios are indicated in Tables 6 and 7. The analyses that included all nonseroconverter cases (without restriction to incident cases) and controls indicated that fewer years of education (O.R.=0.9, 95% CI=0.8, 0.95), tetanus booster in past 2 years (O.R.=2.4, 95% CI=1.2, 4.7), past history of tuberculosis or a positive tuberculin skin test (O.R.= 3.1, 95% CI=1.2,8.0), and autoantibodies, either a positive ANA or rheumatoid factor (O.R.=1.9, 95% CI=1.07, 3.2) were independently associated with an IWB. For males, sex with a prostitute since 1978 (O.R.=4.1, 95% CI=1.3, 12.7), tetanus booster in the past 2 years (O.R.=3.0, 95% CI=1.1,8.1), and prior sharing of needles during intravenous drug use (O.R.=3.6, 95% CI=1.02, 12.9) were independently associated with an IWB. Among females, parity (O.R. 1.2, 95% CI=1.1,1.5), fewer STDs (O.R. 0.4, 95% CI=0.2, 0.8), and fewer years of education (O.R. 0.9, 95% CI=0.8,0.97) were independently associated with an IWB.

Among the incident cases (combining men and women), fewer years of education, autoantibodies, and previous tuberculosis or positive PPD were indepndently associated with an IWB (Table 7). Among incident males, sex with a prostitute, autoantibodies, and tetanus booster in previous two years were associated with an IWB. Among incident females, fewer years of education, fewer STDs, and parity were associated with an IWB.

HLA antibodies were performed for 78 cases; anti-class I HLA reactivity was found in 13 (17%) and no anti-class II HLAreactivity was observed. All but one of the cases who demonstrated anti-class I HLA reactivity were multiparous females.

No cross-reactivity was seen with HTLV-1 in 176 cases tested by HTLV-1 EIA. One additional case had a weakly positive HTLV-1 EIA and an indeterminate HTLV-1 Western blot with a p19 and p29 band. Two cases with travel to or residence in West Africa were tested for HIV-2, one by HIV-2 EIA and RIPA which were negative and one by PCR which was negative.

Psychological Impact of IWB

Cases and controls reported similar rates of previous depression; 57 (37%) of 154 cases and 30 (26%) of 114 controls reported a period of depression lasting two weeks or longer (Table 8). The reported rate of previous psychotropic drug use was also similar between cases and controls; 39 (26%) of cases and 27 (27%) of controls reported having taken antidepressants in the past. The mean score on the 13-item Beck Depression Inventory (BDI) was 4.7 (±5) among cases and 3.7 (±4) among controls (P=NS). The only significant difference between cases and controls was the score on the Symptom Checklist 90 (SCL-90) anxiety scale, in which cases had significantly higher mean self-reported anxiety at the first study visit (P=<0.001).

The univariate odds ratio for a 10-unit change in the SCL-90 standardized anxiety score was 1.48 (95% CI=1.22,2.06; P < 0.001). The univariate odds ratio for the results of the HIV test indicated as the greatest stressor in the prior six months was 49.2 (95% CI=19.8, 122; P < 0.001). In multivariate analyses, the SCL-90 anxiety score was no longer significant (P=0.57) after the HIV test result as greatest stressor in the past six months was entered (OR=45.2, 95% CI= 16.7, 122.7; P<.001), indicating that the higher anxiety among cases was related to the IWB.

In analyses of the impact of the IWB restricted to the cases only, cases who reported any high risk behavior since 1978 had higher mean SCL-90 anxiety scores (67 ±13) compared to the cases without prior risk behavior (mean score 55 ± 14, p=<0.001, Table 9). Although the mean SCL-90 anxiety score for five of the six seroconverters was higher (67 ± 7) than 122 nonseroconverters (59 ± 15), this difference was not significantly different due to the small number of seroconverters. The SCL-90 anxiety scores for the six gravid women who completed the psychological impact forms were slightly lower than the 63 women tested who were not pregnant at the time of the IWB.

Twenty-five (49%) of 51 high-risk cases compared to 10 (10%) of low-risk cases reported that they thought there was a greater than 50% chance that the IWB was related to HIV-1 infection (P<0.001). At the first study visit, 35 (67%) of the high-risk cases and 34 (35%) of the low-risk cases thought about the IWB at least daily (P=<0.001). Only 20 (46%) of 44 high-risk

cases compared to 62 (69%) of 90 low-risk cases indicated that they would be reassured if they did not seroconvert within six months of serologic follow-up ($\underline{P}$=0.005).

CONCLUSIONS

Risk of seroconversion and specificity of supplemental tests

The long-term outcome of persons identified as being repeatedly reactive by screening EIA and indeterminate by Western blot for HIV-1 is not well-characterized. A more rapid determination of HIV-1 infection among such persons through delineation of epidemiologic and serologic characteristics would benefit both patients and clinicians. In this cohort study of 236 adults referred because of prior reactive HIV-1 EIAs and IWB, of whom 159 have been followed for six months or longer by August 1991, we found HIV-1 infection in only 6 (14.7%) of 41 high-risk cases and 0 of 118 low-risk cases ($\underline{P}$ <0.01). Of the 41 high-risk cases, 17 had a p24 band on Epitope blot initially, of whom six seroconverted (35%; 95% CI = 14.2%, 61.7%); none of the 23 high-risk cases with other bands seroconverted (0%; 95% CI = 0%, 14.8%)].

The low risk of seroconversion (3.8%) in our sample population was comparable to that of earlier published studies of blood donors with repeatedly reactive EIAs and IWB, which reported seroconversion rates of 3% to 5% [19,20,21]. As in our study, the seroconverters in the earlier blood donor cohorts had p24 antibodies on initial Western blot and admitted to HIV risk behaviors. A recent study by Jackson and coworkers of 99 Minnesota blood donors with indeterminate HIV-1 blots found no evidence of HIV-1 or HIV-2 infection [39].

During the interval between the first repeatedly reactive EIA and IWB result until enrollment into our study, 82 (52%) of the 159 cases with six months or longer follow-up became nonreactive by EIA, 52 (63%) of whom also were nonreactive by Epitope Western blot. The loss of reactivity on EIA and Western blot was related to the duration of time between initial testing and the first study visit and the number of prior EIAs performed before study enrollment. One explanation for this finding is that these were primarily blood donors who had been tested with earlier generations of less specific HIV-1 EIAs and Western blots. Among the Group 2 and 3 cases followed for six months or longer, the Epitope Western blot results sometines fluctuated between negative and indeterminate; 51% remained indeterminate, 12% were always negative, and the other 33% fluctuated between negative and

indeterminate. Two blood donors had false positive Eptiope blots; one had persistent gp41 and gp120/160 bands with no history of HIV-1 vaccine and the other blood donor had p24 and gp120 bands once. Supplemental tests confirmed the lack of HIV-1 infection in both cases.

Based on our study and the findings of Courouce [40], a nonreactive EIA on a follow-up sample in a low-risk individual with an IWB has a high predictive value for lack of HIV-1 infection, and those individuals do not need further follow-up. Of the 159 cases with six months or longer follow-up, 73 of 82 Group 3 cases had no risk factors for HIV-1 infection who could require no further follow-up by this approach. The remaining 77 (48%) Group 2 and 3 cases still required additional evaluation based on their risk history or persistent EIA reactivity. Supplemental assays which might more quickly identify or exclude HIV infection would be desirable in this large group.

The low number of seroconverters in our study precluded estimation of the sensitivity of supplemental tests and, therefore, the predictive value of a negative test. Nevertheless, the specificities of HIV-1 culture, PCR, ENV 9, CBre3, Syva Microtrak, and serum p24 antigen were 100%, 99.1%, 93.5%, 97.1%, 100%, and 100%, respectively among the 159 nonseroconverters. We found that HIV-1 culture, PCR, and the recombinant envelope assays, Syva Microtrak and CBre3, were the three most useful supplemental assays. Although HIV-1 culture and PCR have excellent specificity and sensitivity in many laboratories, and are reported to be useful in diagnosing the presence or absence of HIV-1 infection [41-44], they are not widely available, currently are technically difficult, and have not been extensively evaluated for sensitivity in this specific context of recently infected individuals with IWB who have not yet seroconverted.

Recombinant envelope assays, such as CBre3 with a specificity of 97.1% or the SYVA Microtrak with a specificity of 100%, might be useful as supplemental tests, providing quantitative and rapid clarification of HIV-1 infection among persons with IWB. Prior studies of CBre3® (Cambridge Biosciences, Boston, MA), have shown high sensitivity in seroconverter panels [33] and excellent negative predictive value in IWB [45]. The high prevalence of indeterminate recombinant HIVAGEN results in our study population

reflected reactivity to one or more gag epitopes. The specificity of HIVAGEN recombinant envelope proteins was comparable to that of CBre3, Syva Microtrak, and ENV 9, but the additional core and polymerase proteins did not help to resolve the IWB patterns.

Although the U. S. Army and other investigators have found RIPA to be a sensitive assay for detecting antibody to HIV-1 envelope glycoproteins compared to Western blot during the course of seroconversion [46], we found weak reactivity to p55 or gp120 in 12 (21%) of the 56 nonseroconverters tested. Additionally, RIPA is a labor-intensive test that requires radiolabeled lysate and is not practical for routine clinical use.

The p24 antigen assay was 100% specific but detected only two of six seroconverters in our series and was negative in 24 seroconverters prior to a diagnostic Western blot in the MACS cohort [42]. A study of p24 antigen screening among male blood donors in the US found the specificity of p24 antigen to be 100%, but the sensitivity was only 11.4% [47].

Because our cohort included individuals with varying intervals from first detection of IWB until study enrollment, the duration of this interval represents a possible confounder, which we attempted to control for in our analyses. After adjusting for the time between the initial IWB and study enrollment, the higher proportion of Group 2 cases compared to Group 3 cases with high-risk sexual partners since 1978 was statistically significant ($\underline{P}$ =<0.001). This suggests that a factor associated with high-risk sexual contact may account for persistent EIA reactivity and IWB. This provocative finding may reflect sampling bias or inadequate controlling for confounding, but warrants further investigation.

Based on our study results, we propose the following algorithm for evaluating individuals with IWB (Figure 1). The first step is to re-evaluate the individual's risk behaviors for possible exposure to HIV-1 and to repeat the EIA. Risk assessment, however, will not always accurately identify individuals with risk behaviors [48]; therefore, our recommendations incorporate both reported history of risk behavior, as well as persistence of the EIA reactivity and the presence or absence of p24 antibodies on Western blot. The proportion of individuals in Groups 1, 2, and 3 will vary according to the time between initial

and repeat HIV-1 testing, HIV risk status of the population tested, and the use of different commercial sources of EIA and Western blot kits at repeat testing.

Repeat EIA and Western blot at one month after the initial IWB will often detect the seroconverters, as was demonstrated in five of the six seroconverters in this sample, and in all 18 seroconverters in the series by Wilbur et al [14]. If the EIA is persistently reactive and the Western blot becomes positive, but infection seems implausible based on the individual's risk history, an EIA and Western blot should be repeated on a subsequent sample. Among those individuals with persistent reactive EIA and IWB who have not seroconverted upon repeat testing one month later, the risk of seroconversion is probably low. Nonetheless, we would recommend that high-risk individuals should be followed for at least six months following their last potential exposure to HIV-1, or longer if they still engage in high-risk behavior with repeat EIAs and Western blots at 3 to 6 month intervals. Horsburgh and colleagues have reported that 50% and 95% of individuals will seroconvert within 3 and 6 months after acquiring infection, respectively [49].

Low-risk individuals with persistent IWB with p24 or envelope bands could be followed for at least three months in case they have denied existing risk behaviors, with repeat EIA and Western blot performed at 3 months. Although the sensitivity of supplemental tests in detecting the infrequent seroconversion in such individuals will be difficult to measure, negative supplemental tests may be useful in reassuring such individuals, especially in situations such as pregnancy and applications for insurance and immigration. Low-risk individuals with bands on Western blot other than p24 antibodies or Western blots that are negative on repeat testing can be reassured that they are not infected and advised that they do not need further serologic follow-up.

High-risk individuals who revert to a negative Western blot or have bands other than p24 or envelope bands could be followed for six months after their last high-risk behavior to exclude seroconversion. Negative supplemental tests may allow cautious reassurance, although again the sensitivity of such tests in this setting is uncertain. The eventual utility of supplemental tests for help in managing such persons with IWB will be determined by

further information from clinical epidemiologic studies that assess the sensitivity and predictive value of supplemental tests. The etiology of false positive supplemental test results, like the etiology of IWB, requires further study.

Risk factors for indeterminate HIV-1 Western blots

The multivariate analyses indicate that several factors may be independently associated with IWB among the nonseroconverters. In the analyses in which men and women cases and controls were compared as a group, prior tuberculin positivity or tuberculosis history, a tetanus booster in the past 2 years, and autoantibodies (either a positive ANA or rheumatoid factor) were associated with an IWB. Although recall bias could influence cases reporting a higher prevalence of past positive PPD, it is unlikely to fully account for the higher prevalence among cases. Mycobacterial diseases, such as leprosy or TB, have been associated with biologic false syphilis serologies. A recent tetanus booster could result in immunogenic stimulation of B cells and nonspecific antibody production that could result in cross-reactivity with epitopes or proteins on the HIV-1 Western blot.

Autoantibodies, such as a positive ANA or rheumatoid factor, might indicate that such individuals have antibodies against some of their own cellular proteins, which could cross-react with cellular proteins or epitopes from the H-9 or CEM cells in which HIV-1 is cultured to produce HIV-1 Western blots. The cases with autoantibodies usually had low titer ANAs (median tier of 40, range 10-640) or rheumatoid factor (median titer of 320, range 20-2560) and the majority had no symptoms or prior diagnosis of autoimmune illness. We found no significant association between autoantibodies and parity or presence of p24 or p17 bands on Western blot. Although a link between human retroviruses and certain autoimmune illnesses has been suggested, limited data exist to support this hypothesis. Talal and colleagues have reported reverse transcriptase activity and electron micrographs of a possible Type C intracisternal retrovirus isolated from two of six patients with Sjogren's disease [50]. These findings await confirmation by other investigators. A high proportion of p24 reactivity has been reported among persons with systemic lupus erythematosus [51,52].

An initial report of a possible retroviral etiology for hyperthryoidism due to Grave's disease [53], has been refuted by other investigators [54].

Men and women may differ in the risk factors associated with IWB. The strongest risk factors for IWB among males were contact with a prostitute and sharing of needles during intravenous drug use which are associated with transmission of HIV-1 and other infectious diseases, as well as autoantibodies and a tetanus booster in the past 2 years. In contrast, the risk factors for women were parity, fewer years of education, fewer STDs. Fewer years of education and fewer STDs among the female cases than controls may reflect sampling of higher risk controls by testing site. Parity could operate as a risk factor for IWB due to alloimmunization during pregnancy with cross-reactivity to cellular proteins that comigrate with the Western blot. Although there was a trend towards current pregnancy as a risk factor for IWB among incident female cases, 13 of whom were currently pregnant at the time they were detected as indeterminate by HIV-1 Western blot, the sample size was not large enough for this variable to reach statistical significance.

The results of the conditional logisitic regressions did not differ significantly when the cases were restricted to "incident" cases who had first tested indeterminate in the six months prior to study enrollment, indicating that misclassification is not apparently a significant problem in including all cases in the regression analyses.

Psychological impact of IWB

The cases reported higher anxiety than the controls at the first study visit ($P < 0.001$), but reported comparable rates of previous depressive episodes lasting two weeks or longer, previous antidepressant and other psychotropic drug use, and had similar scores on the 13-item Beck Depression Index. The greater anxiety among the cases at the first study visit was accounted for by the IWB result, as indicated by logistic regression in which the difference in anxiety between cases and controls persisted after entering other significant variables by univariate analysis, but became nonsignificant when the IWB as major life stressor on the life event scale was entered into the regression equation with the SCL-90 anxiety score.

The high-risk cases had higher anxiety scores than the low-risk cases ($\underline{P}$ <0.001), and the mean SCL-90 scores of seroconverters was higher than the nonseroconverters but the number of seroconverters was too few for that difference to reach statistical significance. A higher proportion of high-risk cases reported thinking the IWB was related to HIV-1 infection, thinking about the IWB at least daily, and indicated that they would not feel reassured even if they did not seroconvert in six months follow-up.

Common concerns raised by cases included questions about whether they were infected with HIV-1; whether they could have been unknowingly exposed to HIV-1 by previous sexual partners, blood transfusions, health care, or other means; whether they would remain indeterminate by HIV-1 serologies; what the potential impact would be on their relationships, and their eligibility to donate blood, participate in an in vitro fertilization program, be insured, immigrate, obtain travel visas, and be awarded custody; whether they have some other medical condition that caused the IWB; and how quickly could they find out whether they were infected with HIV.

At the end of new enrollments into the study in July 1991, we designed a brochure about IWBs to address these concerns which would be sent out by virology laboratories in Washington state with IWB results (Appendix C). One brochure was written for health care providers with technical information, our proposed algorithm for follow-up of persons with IWB, and a brief reference list. The purpose of the provider brochure was to provide information on etiologies and follow-up recommendations for providers who often have had little experience in counseling persons with IWB. The second brochure was written in question-answer format with simple language to be given to the persons with IWB. The purpose of the patient brochure was to provide information about HIV serologic tests, the overall low rate of seroconversion among persons with IWBs, other possible etiologies of IWBs, and recommendations for follow-up. These brochures will be available as resources after our study enrollment is completed and hopefully will address provider and patient concerns about the significance and impacts of indeterminate HIV-1 Western blots.

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TABLE 1

DEMOGRAPHIC AND HIV RISK FACTORS OF CASES , THEIR SEXUAL PARTNERS, AND EIA NEGATIVE CONTROLS:

	CASES	CONTROLS	CASES' SEX PARTNERS	
NUMBER OF CASES	236	144	79	p-value
AGE				
Median (range)	37 (13,85)	35 (17,69)	37.5 (19,62)	NS
SEX				
Male (%)	112 (47%)	68 (47%)	42 (53%)	NS
RACE				
Caucasian (%)	209 (89%)	125 (87%)	72 (91%)	NS
MARITAL STATUS				
Never married (%)	77 (33%)	60 (42%)	13 (17%)	<0.001
Married (%)	107 (45%)	58 (40%)	57 (72%)	
Divorced/widow (%)	51 (22%)	26 (18%)	9 (11%)	
EDUCATION				
Median in yrs (range)	14 (0,23)	16 (5,32)	14 (10,22)	0.003
ANNUAL FAMILY INCOME				
Greater than $20,000	135 (61%)	75 (54%)	52 (72%)	0.04
SEXUAL PREFERENCE				
Heterosexual (%)	208 (90%)	120 (85%)	75 (95%)	NS
Bisexual (%)	9 (4%)	7 (5%)	2 (3%)	
Homosexual (%)	13 (6%)	12(9%)	2 (3%)	
Never sexually active (%)	2 (1%)	3 (2%)	0 (0%)	
NUMBER OF SEXUAL PARTNERS				
Median, past 3 months	1 (0,90)	1 (0,72)	1 (0,2)	.003
Median, past year	1 (0,300)	1 (0,121)	1 (0,6)	NS
PAST STDs	82 (35%)	64 (44%)	13 (16%)	<.001
HIGH-RISK SEXUAL PARTNER				
Since 1978 (%)	70 (30%)	42 (30%)	15 (19%)	.16
HIV+ SEXUAL PARTNER	9 (4%)	3 (2%)	0 (0%)	.16
PAST PROSTITUTION	11 (5%)	3 (2%)	1 (1%)	NS
TRANSFUSION 1978-85	11 (5%)	4 (3%)	4 (5%)	NS
PAST IV DRUG USE	21 (9%)	8 (6%)	5 (6%)	NS
HEMOPHILIA	1 (0.4%)	1 (.7%)	1 (1%)	NS
ANY RISK FOR HIV	78 (33%)	45 (32%)	22 (28%)	NS

TABLE 2

CHARACTERISTICS OF 159 CASES FOLLOWED FOR > 6 MONTHS

	GROUP 1* EIA and WB positive	GROUP 2 Still reactive EIA	GROUP 3 No longer reactive EIA	p-value
NUMBER OF CASES	6	71	82	
AGE				
Median in yrs (range)	34 (22,58)	35 (16,68)	42 (17,70)	NS
SEX				
Male (%)	5 (83%)	27 (38%)	45(55%)	0.03
RACE				
Caucausian (%)	6 (100%)	63 (89%)	79 (96%)	NS
MARITAL STATUS				
Never married (%)	1 (17%)	30 (42%)	17(21%)	<0.001
Married	0 (0%)	32 (45%)	48 (59%)	
Divorced/separated	5 (83%)	7 (10%)	16 (20%)	
EDUCATION				
Median in yrs (range)	15 (13,22)	14 (4,22)	15 (8,23)	NS
ANNUAL FAMILY INCOME				
Above $20,000 (%)	4 (67%)	34 (53%)	64 (81%)	0.002
MEDIAN NUMBER OF SEXUAL PARTNERS				
Past 3 months (range)	2 (0,90)	1 (0,20)	1 (0,4)	NS
Past year	5 (0,300)	1 (0,100)	1 (0,15)	0.07
SEXUAL PREFERENCE				
Heterosexual (%)	1 (17%)	63 (88%)	75 (91%)	<0.001
Bisexual	1 (17%)	4 (6%)	2 (2%)	
Homosexual	4 (67%)	4 (6%)	3 (4%)	
Never sexually active	0 (0%)	0 (0%)	2 (2%)	

HIGH-RISK SEXUAL PARTNER SINCE 1978				
	6 (100%)	23 (32%)	6 (7%)	<0.001
PAST SEXUALLY TRANSMITTED DISEASES**				
	4 (67%)	21 (30%)	19 (24%)	NS
HISTORY OF PROSTITUTION	2 (33%)	2 (3%)	0 (0%)	<0.001
INTRAVENOUS DRUG USE	1 (17%)	4 (6%)	2 (2%)	NS
BLOOD PRODUCT TRANSFUSION, 1978-85				
	0 (0%)	4 (6%)	4 (5%)	NS
HEMOPHILIA	0 (0%)	0 (0%)	1 (1%)	NS
ANY HIGH RISK SINCE 1978	6 (100%)	26 (37%)	9 (11%)	<0.001
TIME BETWEEN INITIAL IWB AND STUDY VISIT ONE				
Median in months (range)	1 (0.5, 3)	3 (0.5,51)	13 (0.5,55)	<0.01

LEGEND FOR TABLE 2:

* All cases were referred because of a past repeatedly reactive EIA assays and one or more IWB prior to the first study visit. Group 1 includes the six individuals who seroconverted during the study period at times ranging from one to ten months. Groups 2 and 3 are defined by the results of the HIV-1 EIA at the first study visit. At the first study visit, Group 2 cases were still repeatedly reactive (RR) on EIA and Group 3 cases were nonreactive (NR) by EIA.

** Sexually-transmitted diseases included genital herpes, gonorrhea, chlamydial infection, genital warts, genital ulcerations, and hepatitis B.

TABLE 3

RESULTS OF HIV-1 EIA AND WESTERN BLOTS AT FIRST STUDY VISIT

--in cases, controls, cases' sexual partners--

	CASES	CONTROLS	CASES' SEXUAL PARTNERS
NUMBER OF CASES	236	144	79
EIA			
Repeatedly reactive (%)	132 (56%)	117 (98%)	5 (6%)
EPITOPE WESTERN BLOT			
Negative	83 (35%)	96(77%)	56 (74%)
Indeterminate	147 (62%)	29(23%)	17 (22%)
p17*	65	6	2
p24*	71	11	1
other bands*	33	12	12
Positive**	5 (2%)	0 (0%)	3 (4%)
DUPONT WESTERN BLOT			
Number of tests performed***	145	77	56
Negative	23 (16%)	58 (75%)	30 (54%)
Indeterminate	118 (81%)	19 (25%)	24 (43%)
p17*	51	1	1
p24*	76	14	15
other bands*	24	4	7
Positive**	5 (3%)	0 (0%)	2 (4%)

LEGEND FOR TABLE 3:

*The percentages for the indeterminate banding patterns may add up to greater than 100% because persons could have more than one band present (eg., p17 and p24, p24 and p55).

** Five of the six seroconverters had a positive Western blot at the first study visit; all six seroconverters had been referred with a p24 band on initial blot. The sixth case seroconverted 10 months after his initial Western blot showed a p24 band only with ongoing risk behavior during the study period. Western blots were interpreted as positive using CDC interpretative criteria (if at least two of the following anti-HIV antibodies were present: p24, gp41, and/or gp120/160).

***Dupont Western blots were performed in a subset of study subjects.

TABLE 4

EPITOPE HIV-1 WESTERN BLOT RESULTS IN 159 CASES FOLLOWED ≥ 6 MONTHS

-- at first study visit after enrollment--

	GROUP 1*	GROUP 2	GROUP 3
	EIA and WB positive	EIA still reactive	EIA no longer reactive
NUMBER OF CASES	6	71	82
NEGATIVE--Total (%)	1 (17%)	15 (21%)	52 (63%)
		|—p<0.001—|	
INDETERMINATE--Total (%)	1 (17%)**	54 (76%)	30 (37%)
p17 only	0	26	1
p24 only	1	19	14
p17 & p24	0	5	1
pol bands	0	2	6
POSIT.VE***--Total (%)	4 (67%)	2 (3%)****	0 (0%)

LEGEND FOR TABLE 4:

*All cases were referred because of a past repeatedly reactive EIA assays and one or more IWB prior to the first study visit. Group 1 includes the six individuals who seroconverted during the study period at times ranging from one to ten months. Groups 2 and 3 are defined by the results of the HIV-1 EIA at the first study visit. At the first study visit, Group 2 cases were still repeatedly reactive (RR) on EIA and Group 3 cases were nonreactive (NR) by EIA.

**Five of the six seroconverters had a positive Western blot at the first study visit; all six seroconverters had been referred with a p24 band on initial blot. The sixth case seroconverted 10 months after his initial Western blot showed a p24 band only with ongoing risk behavior during the study period.

***Western blots were interpreted as positive using CDC interpretative criteria (if at least two of the following anti-HIV antibodies were present: p24, gp41, and/or gp120/160).

****Two low-risk cases had envelope bands on Western blot (eg., p24 and gp160), one of which persisted with the same band intensity and pattern on repeat blood draws, and in the other case the gp160 band was not present on subsequent blood draws. Both cases have had negative polymerase chain reaction and recombinant tests and the positive Western blots were considered false positives.

TABLE 5

SPECIFICITY OF SUPPLEMENTAL HIV TESTS

-in 153 subjects followed ≥ 6 months who did not develop positive blots-

	GROUP 2* EIA still reactive		GROUP 3* EIA no longer reactive	
	high risk (N = 26)	low risk (N = 45)	high risk (N = 9)	low risk (N=73)
HIV CULTURE				
negative	17/17	28/28	8/8	32/32
POLYMERASE CHAIN REACTION				
negative	22/22	37/37	6/7	46/46
indeterminate	-	-	1/7**	-
SERUM p24 ANTIGEN				
negative	17/17	28/28	6/6	26/26
ENV 9 EIA				
negative	13/14	22/22	3/4	21/22
low positive	1/14	-	1/4	1/22
HIVAGEN EIA				
negative	1/15	0/26	2/8	22/36
indeterminate	14/15	26/26	6/8	12/26
SYVA MICROTRAK				
negative	5/5	10/10	5/5	33/33
CBre3 EIA				
negative	8/8	12/13	2/2	11/11
positive	-	1/13	-	-
RADIOIMMUNOPRECIPITATION				
negative	10/13	17/22	5/6	12/15
indeterminate	3/13	5/22	1/6	3/15

LEGEND FOR TABLE 5:

* All cases were referred because of a past repeatedly reactive EIA assays and one or more IWB prior to the first study visit. Group 1 includes the six individuals who seroconverted during the study period at times ranging from one to ten months. Groups 2 and 3 are defined by the results of the HIV-1 EIA at the first study visit. At the first study visit, Group 2 cases were still repeatedly reactive (RR) on EIA and Group 3 cases were nonreactive (NR) by EIA.

** Subsequent testing did not confirm the initial positive PCR result.

*** The three cases with positive recombinant ENV 9 results had borderline specimen to cut-off optical density values of 1.1 and 1.4.

FIGURE 1

PROPOSED ALGORITHM FOR INDETERMINATE HIV-1 WESTERN BLOTS

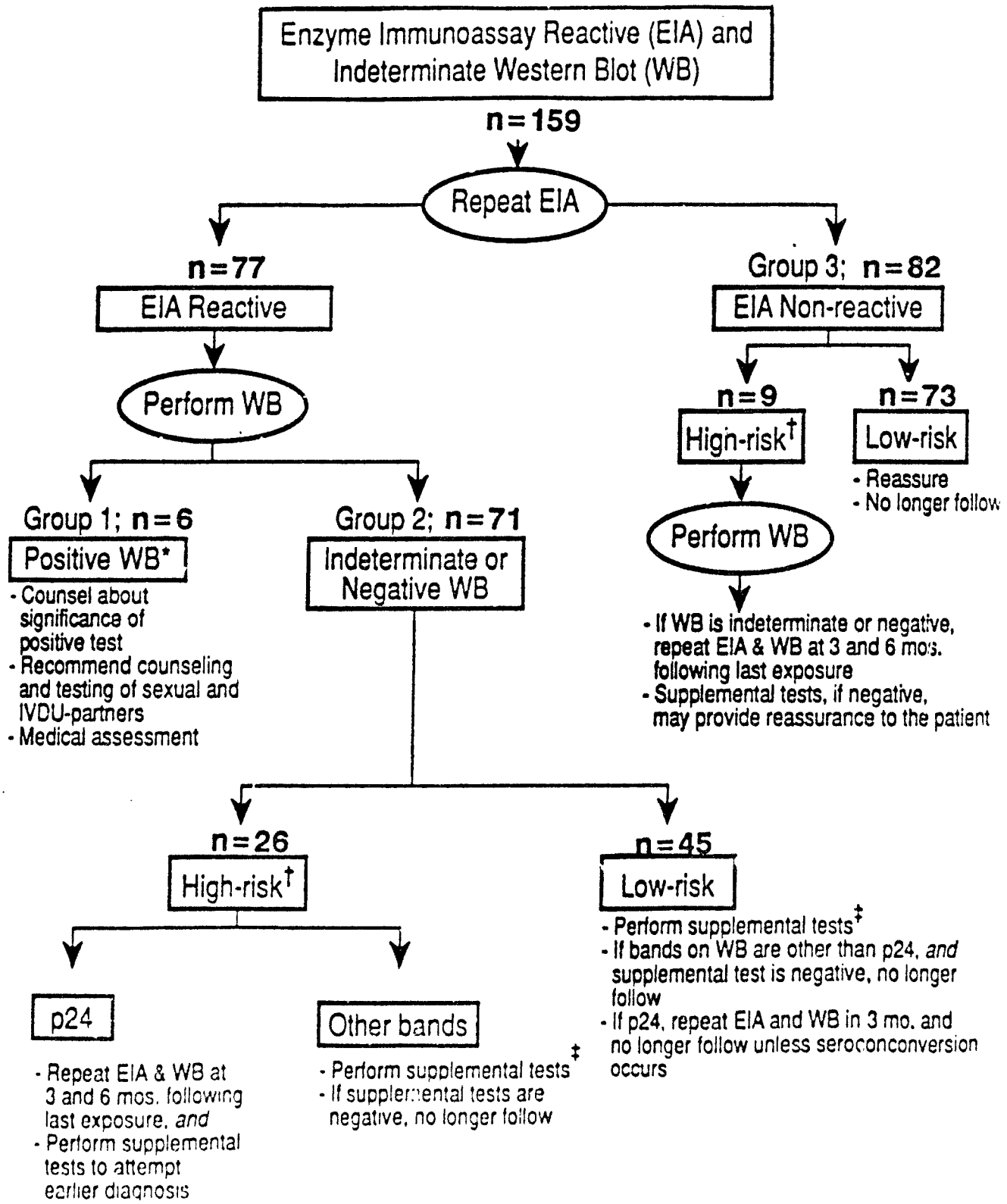


TABLE 6

RISK FACTORS FOR INDETERMINATE HIV-1 WESTERN BLOTS

--NONSEROCONVERTER CASES AND CONTROLS--

RISK FACTOR N (%)	CASES N=230	CONTROLS N=144	UNIVARIATE ODDS RATIO	MULTIVARIATE ODDS RATIO	Multivariate p-value
Education (median,range)	14 (0,23)	15 (5,32)	0.9	0.9 (0.8, 0.95)	<.001
Tetanus booster past 2 yrs	43 (19%)	12 (9%)	2.2	2.4 (1.2, 4.7)	.011
Past + PPD*	25 (11%)	4 (3%)	2.7	3.1 (1.2, 8.0)	.021
Autoantibodies**	69 (34%)	24 (22%)	1.9	1.9 (1.07, 3.2)	.027
Past STDs***	77 (35%)	60 (49%)	0.6	–	
Sex w/prostitute	22 (9%)	5 (4%)	2.7	–	

--MALE CASES (nonseroconverters) AND CONTROLS--

RISK FACTOR N (%)	CASES N=107	CONTROLS N=68	UNIVARIATE ODDS RATIO	MULTIVARIATE ODDS RATIO	Multivariate p-value
Sex w/prostitute	22 (9%)	4(7%)	3.9	4.1 (1.3, 12.7)	.01
Tetanus past 2 yrs	24 (22%)	6 (9%)	3.0	3.0 (1.1, 8.1)	.03
Shared needles	15 (14%)	4 (7%)	3.4	3.6 (1.02, 12.9)	.046
Past + PPD*	12 (11%)	1 (2%)	4.5	–	
Autoantibodies	31 (32%)	10 (17%)	2.3	–	

--FEMALE CASES (nonseroconverters) AND CONTROLS--

RISK FACTOR N (%)	CASES N=122	CONTROLS N=75	UNIVARIATE ODDS RATIO	MULTIVARIATE ODDS RATIO	Multivariate p-value
Parity	91 (75%)	39 (52%)	1.3	1.2 (1.1,1.5)	.009
Past STDs***	33 (29%)	66 (53%)	0.3	0.4 (.2,.8)	.007
Education	14 (0,22)	16 (9,32)	0.8	0.9 (.8, .97)	.01
Currently pregnant	14 (12%)	3 (4%)	3.3	–	

LEGEND FOR TABLE 6:

To assess risks for indeterminate HIV-1 Western blots (IWB) other than acute HIV-1 infection, the risk factors for IWB were compared between the nonseroconverter casss and the EIA negative controls. Conditional logistic regression was performed with three strata of cases and controls--blood donors, high-risk cases, and women's and prenatal clinics. Low-risk cases for whom random controls could not be enrolled (i.e., life insurance applicants and patients referred by private MDs) were matched with blood donor controls.

*PPD=purified protein derivative, a skin test to detect past exposure to tuberculosis

**Autoantibodies indicates either a positive antinuclear antibody (ANA) and/or rheumatoid factor.

***Sexually-transmitted diseases included genital herpes, gonorrhea, chlamydial infection, genital warts, genital ulcerations, and hepatitis B.

TABLE 7

RISK FACTORS FOR INDETERMINATE HIV-1 WESTERN BLOTS

--INCIDENT* CASES (nonseroconverters) AND CONTROLS--

RISK FACTOR N (%)	CASES N=156	CONTROLS N=144	UNIVARIATE ODDS RATIO	MULTIVARIATE ODDS RATIO	Multivariate p-value
Education (yrs)	14 (0,23)	15 (5,32)	0.9	0.9 (.8,.96)	.004
Autoantibodies**	46 (32%)	25 (19%)	2.0	2.0 (1.1, 3.6)	.022
Past + PPD***	18 (12%)	6 (4%)	3.2	3.1 (1.1,8.3)	.024
Sex w/prostitute	18 (12%)	6 (4%)	2.8	–	
Tetanus booster past 2 yrs	29 (19%)	15 (10%)	1.9	–	

--INCIDENT MALE CASES (nonseroconverters) AND CONTROLS--

RISK FACTOR N (%)	CASES N=70	CONTROLS N=68	UNIVARIATE ODDS RATIO	MULTIVARIATE ODDS RATIO	Multivariate p-value
Sex w/prostitute	17 (24%)	5 (7%)	4.2	6.5 (1.7, 24.7)	.006
Autoantibodies	22 (34%)	10 (17%)	2.5	2.7 (1.1, 6.4)	.03
Tetanus booster	17 (24%)	6 (9%)	3.1	3.2 (1.01, 9.9)	.048
HIV risk behavior since 1978	36 (51%)	22 (32%)	3.0	–	
Past + PPD	10 (14%)	2 (3%)	9.8	–	
Shared needles	13 (19%)	4 (6%)	4.1	–	

--INCIDENT FEMALE CASES (nonseroconverter) AND CONTROLS--

RISK FACTOR N (%)	CASES N=86	CONTROLS N=76	UNIVARIATE ODDS RATIO	MULTIVARIATE ODDS RATIO	Multivariate p-value
Education	14 (0,22)	16 (9,32)	0.8	0.85 (.7, .97)	.01
Past STDs****	31 (37%)	37 (49%)	0.4	0.4 (.2,.9)	.02
Parity	65 (76%)	39 (51%)	1.3	1.2 (1.03,1.5)	.02
Currently pregnant	13 (16%)	3 (5%)	4.2	–	

LEGEND FOR TABLE 7:

To assess risks for indeterminate HIV-1 Western blots (IWB) other than acute HIV-1 infection, the risk factors for IWB were compared between the nonseroconverter casss and the EIA negative controls. Conditional logistic regression was performed with three strata of cases and controls--blood donors, high-risk cases, and women's and prenatal clinics. Low-risk cases for whom random controls could not be enrolled (i.e., life insurance applicants and patients referred by private MDs) were matched with blood donor controls.

*Incident cases were defined as those cases whose initial indeterminate HIV-1 Western blot was six months or less prior to study enrollment.

**Autoantibodies indicates either a positive antinuclear antibody (ANA) and/or rheumatoid factor.

***PPD=purified protein derivative, a skin test to detect past exposure to tuberculosis

****Sexually-transmitted diseases included genital herpes, gonorrhea, chlamydial infection, genital warts, genital ulcerations, and hepatitis B.

TABLE 8

ANXIETY AND DEPRESSION AMONG CASES AND CONTROLS

--Reported at first study visit--

	CASES N=154	CONTROLS N=114	p-value
Past history of depression	57 (37%)	30 (26%)	NS
Past use of psychotropic meds	39 (26%)	27 (27%)	NS
SCL-90 anxiety subscale*			
mean (± SD)	59 (± 15)	51 (±13)	<.001
25% quartile	48	39	
50% quartile	62	49	
75% quartile	70	61	
Beck Depression Inventory**			
Mean (±SD)	4.7(± 5)	3.7 (± 4)	NS

* The SCL-90 anxiety scale was standardized to nonpsychiatric outpatients ; the minimum score is 36 for males and 38 for females [38].

**The 13-item Beck Depression Inventroy (BDI) was used. The estimated degree of depression according to the BDI score is 0-4=none or minimal, 5-7=mild, 8-15=moderate, and 16+=severe [35-37].

TABLE 9

ANXIETY ABOUT IWB AT FIRST STUDY VISIT

--Subgroup analyses among cases--

	SCL-90* Anxiety scale Mean (± SD)	p-value
High risk behavior since 1978 (n=48)	67 (± 13)	<.001
No risk behavior since 1978 (n=88)	55 (± 14)	
Seroconverters (n=5**)	67 (± 7)	NS
Nonseroconverters (n=131)	59 (±15)	
Gravid women (n=6**)	55 (± 14)	NS
Nongravid women (n=63)	58 (± 14)	

	High-risk cases N=55**	Low-risk cases N=99**	p-value
Thinks ≥ 50% chance IWB* is related to HIV	25/51 (49%)	10/97 (10%)	<.001
Thinks about IWB* daily	35/52 (67%)	34/96 (35%)	.002
Reassured if doesn't seroconvert in 6 mos	20/44 (46%)	62/90 (69%)	.005

LEGEND FOR TABLE 9:

* The SCL-90 anxiety scale was standardized to nonpsychiatric outpatients ; the minimum score is 36 for males and 38 for females [38].

** Not all cases filled out all items on the psychological questionnaire. Percentages are computed based on the total respondents for each item.

APPENDIX A

INDETERMINATE WESTERN BLOT STUDY

ID RECORD (REV891030)

ID.0	Subject ID	_ _ _ _
ID.0A	Subject Status 0=case; 1=control; 2-9=case's SP	_
ID.0B	Referral Site 01=APP;02=PSBC;03=TacBC;04=Health Dept; 05=STD;06=PP;07=UW/HMC Women's Clinic; 08=Methadone Clinic;09=TB Clinic; 10=Immigration;11=PMD;12=Portland Red Cross; 13=Military;14=Insurance;	_ _
ID.0C	Enrollment Date	_ _ _ _ _ _
ID.1	DEMOGRAPHICS	
ID.1A	Sex 1=M;2=F	_
ID.1B	Marital Status 1=Never married;2=Married;3=Divorced 4=Widowed;5=Separated	_
D.1C	Race 1=Cauc;2=Blk;3=NAI;4=Hisp;5=Asian;6=Other	_
D.1D	Years of education	_ _
D.1E	Occupation 1=Health Care;2=Agric;3=Prof; 4=Clerical;5=Manuf;6=Admin 7=Other:specify_____	_
D.1F	Gross family income Code 0-9 corresponding to 1st digit of ten thousand ex: <10,000=0;50,000=5;>90,000=9	_
D.1G	Number of people supported	_
D.1H	Census tract	_
	Write in address & zipcode_____ _____	

ID.1I	Birthdate	_ _ _ _ _ _
ID.1J	Birthplace 0=Foreign born;1=AK,HI,WA,OR,CA; 2=MT,ID,WY,NV,UT,CO,AZ,NM;3=ND,SD,NE,KS,MN,IA,MO; 4=OK,TX,AR,LA;5=WI,IL,MI,IN,OH;6=KY,TN,MS,AL; 7=NY,NJ,PA;8=ME,VT,NH,MA,RI,CT; 9=WV,MD,DE,DC,VA,NC,SC,AL,GA,FL	_
ID.1K	FAMILY HX	
ID.1Ka	Autoimmune disease	_
ID.1Kb	Hemophilia	_
ID.1L	LIVED IN HIGH RISK AREA IN p10y	
ID.1La	San Francisco	_
ID.1Lb	New York/New Jersey	_
ID.1Lc	Miami	_
ID.1M	TRAVELED TO FOREIGN HIGH RISK AREA p5y	
ID.1Ma	Central Africa	_
ID.1Mb	Haiti	_
ID.2	HIV RISK FACTORS	
ID.2A	Reason for HIV screen 0=blood donor;1=considering pregnancy; 2=new SP;3=concern over p sexual exposure; 4=past IVDU;5=is pregnant;6=life insurance; 7=military/immigration screen;8=HC worker c exp; 9=other_____	_
ID.2B	Secondary reason for HIV screen Use ID.2A codes	_
ID.2C	Sexual preference 1=homosexual;2=bisexual;3=heterosexual	_
ID.2D	SP evaluated 0=none;1=SP refuses; 2=SP will participate	_
MALES:		
ID.2E	Had vasectomy	
ID.2F	Been circumcized	

INDETERMINATE WESTERN BLOT STUDY

BEHAVIORAL HISTORY (REV891030)

BE.0	Subject Id		____		
BE.0A	Visit Number	1	3	6	9
BE.0B	Visit Date	______	______	______	______
BE.1	MISC HISTORY				
	Have you ever: 0=no;1=yes;9=unk;.=ND				
BE.1A	Had tatoo/acu	_	_	_	_
BE.1Aa	# mo 1st tat/acu	___	___	___	___
BE.1B	Raw dairy prod p5y	_	_	_	_
BE.2	ANIMAL EXPOSURE				
BE.2A	Farm animals	_	_	_	_
BE.2Aa	# months	___	___	___	___
BE.2B	Owned a cat 0=no; 1=yes, healthy 2=yes, sick..... 3=yes, hx of Felv shots	_	_	_	_
BE.2Ba	Cat wound ply 0=no; 1=scratched only; 2=bitten only; 3=both	_	_	_	_
BE.2Bb	Owned other pets 0=no;1=dog; 2=rodent;3=other	_	_	_	_

BE.3	SMOKING HISTORY				
BE.3A	Smoked >100 cig LT	_	_	_	_
BE.3Aa	Currently smoke	_	_	_	_
BE.3Ab	Average # cig/day	_ _	_ _	_ _	_ _
BE.3Ac	# month's smoked	_ _ _	_ _ _	_ _ _	_ _ _
BE.3Ad	# month's quit	_ _ _	_ _ _	_ _ _	_ _ _
BE.4	DRINKING HISTORY				
BE.4A	Hx of ETOH prob 0=no; 1=yes,practicing 2=yes, recovering	_	_	_	_
BE.4Aa	Ave # drinks/wk	_ _ _	_ _ _	_ _ _	_ _ _
BE.5	DRUG USE HISTORY				
BE.5A	Marijuana use p5y	_	_	_	_
BE.5Aa	Ave #x's/wk	_ _	_ _	_ _	_ _
BE.5B	Crack use p5y	_	_	_	_
BE.5Ba	Ave #x's/wk	_ _	_ _	_ _	_ _
BE.6	SEXUAL HISTORY				
BE.6A	Age 1st IC c F	_ _	_ _	_ _	_ _
BE.6Aa	# F SP L/T	_ _	_ _	_ _	_ _
BE.6Ab	# F SP p1y	_ _	_ _	_ _	_ _
BE.6Ac	# female SP p3m	_ _	_ _	_ _	_ _
BE.6Ad	# new F-SP p3m	_ _	_ _	_ _	_ _
BE.6B	Age 1st act c male	_ _	_ _	_ _	_ _
BE.6Ba	# M SP L/T	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
BE.6Bb	# M SP p1y	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc	# male SP p3m	_ _	_ _	_ _	_ _
BE.6Bd	# new M-SP p3m	_ _	_ _	_ _	_ _

BE.6Bc	Specific activities p3m				
BE.6Bc1	#x's PV s condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc2	#x's PV c condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc3	Ever had anal IC	_	_	_	_
BE.6Bc4	#x's PR s condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc5	#x's PR c condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc6	#x's OV s dam	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc7	#x's OV c dam	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc8	#x's OP s condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc9	#x's OP c condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc10	#x's IC c menses	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc11	#x's spermicidals	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc12	#x's diaphragm	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc13	#x's cervical cap	_ _ _	_ _ _	_ _ _	_ _ _
BE.7A	Sex with:				
BE.7Aa	Homo/Bisexual male	_	_	_	_
BE.7Ab	IVDU	_	_	_	_
BE.7Ac	Male prostitute	_	_	_	_
BE.YAd	Female prostitute	_	_	_	_
BE.7Ae	Person HIV area	_	_	_	_
BE.7Af	Person with ARC	_	_	_	_
BE.7Ag	Person with AIDS	_	_	_	_
BE.7Ah	HIV + person	_	_	_	_
BE.8	HX OF:				
BE.8A	Hemophilia 0=no;1=Hemophilia A 2=Hemophilia B; 3=Von Wildebrand's Date of dx.....	_	_	_	_
BE.8Aa	Uses FF 0=no;1=currently; 2=prior to 1985	_	_	_	_
BE.8Aa1	#x's/yr	_ _	_ _	_ _	_ _

BE.8Ab	Uses cryo 0=no;1=currently; 2=prior to 1985	_	_	_	_
BE.8Ab1	#x's/yr	_ _	_ _	_ _	_ _
BE.8B	Transfusion 1978 0=no;1=yes	_	_	_	_
BE.8Ba	# units	_	_	_	_
BE.8Bb	Date of transfusion (MMYY)	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
BE.8C	Worked as a prostitute When_____	_	_	_	_
BE.8D	IVDU	_	_	_	_
BE.8Da	Date last used (MMDDYY)	_ _ _ _ _ _	_ _ _ _ _ _	_ _ _ _ _ _	_ _ _ _ _ _
BE.8Db	Shared needles	_	_	_	_
BE.8Dc	Specific drug used 1=cocaine;2=heroin;3=other	_	_	_	_
BE.8Dd	Most common IVDU 1=cocaine; 2=heroin; 3=other	_	_	_	_
BE.8A	Any high risk	_	_	_	_

INDETERMINATE WESTERN BLOT STUDY

MEDICAL HISTORY (REV891024)

ME.0	Subject ID		____		
ME.0A	Visit Number	1	3	6	9
ME.0B	Visit Date	______	______	______	______
ME.1	PAST MEDICAL HISTORY 0=no;1=yes;9=unk;.=ND				
ME.1A	Allergies 0=no;1=yes, not desens 2=yes, desens	_	_	_	_
ME.1B	Surgery 0=no;1=yes	_	_	_	_
ME.1C	Hospitalizations 0=no,1=yes Specify	_	_	_	_ .....
ME.1D	Current meds 0=no;1=yes Specify	_	_	_	_ .....
ME.1E	DC'd meds p3m 0=no;1=yes Specify	_	_	_	_ .....
ME.1F	Viral illnesses p3m 0=no;1=URI;2=LRI; 3=gastroenteritis; 4=other..... Date of illness	_	_	_	_ .....
ME.1G	Depression ≥ 2 wk 0=no;1=yes	_	_	_	_
ME.1Ga	Date dx (MMYY)	____	____	____	____
ME.1Gb	Rx antidepressants 0=no;1=yes	_	_	_	_
ME.1Gc	Rx psychotherapy 0=no;1=yes	_	_	_	_

ME.1I	Autoimmune dis 0=no;1=RA;2=Lupus; 3=thyroiditis;4=Addison's 5=Cushing's;6=other	_	_	_	_
ME.1J	Diabetes 0=no;1=IDDM;2=NIDDM Date of dx.....	_	_	_	_
ME.1K	Tuberculosis 0=no;1=+PPD; 2=hx of rx'd TB	_	_	_	_
ME.1L	Liver disease 0=no;1=hepatitis 2=cirrhosis;3=alcohol; 4=other..... Date of dx.....	_	_	_	_
ME.1M	Cancer 0=no;1=yes..... Date of dx.....	_	_	_	_
ME.1N	Zoster 0=no;1=single dermatome 2=mult dermatomes Date of dx.....	_	_	_	_
ME.1O	Skin diseases 0=no;1=seborrhea; 2=recurrent staph; 3=psoriasis; 4=vitiligo 5=other..... 6=multiple	_	_	_	_
ME.1P	Mononucleosis 0=no;1=presumptive; 2=+monospot	_	_	_	_
ME.1Q	Immunizations p2y 0=no;1=tetanus; 2=pneumococcal 3=Hepatitis B; 4=other	_	_	_	_
ME.1Qa	# mo	_ _	_ _	_ _	_ _
ME.1R	Gamma glob p2y 0=no;1=yes	_	_	_	_
ME.1Ra	# mo g globulin	_ _	_ _	_ _	_ _

FEMALES:

ME.2A	# of pregnancies	_	_	_	_
ME.2Aa	# of deliveries	_	_	_	_
ME.2Ab	# of SAB	_	_	_	_
ME.2Ac	# of TOP	_	_	_	_
ME.2B	Currently pregnant	_	_	_	_
ME.2Ba	# months gestation	_	_	_	_
ME.2C	Rhogam injection 0=no;1=yes	_	_	_	_
ME.2Ca	# mo since Rhogam	_ _	_ _	_ _	_ _
ME.2D	Abnl PAP 0=no;1=ABNL	_	_	_	_
ME.2Da	Date last abnl (MMYY)	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.2E	SP had vasectomy	_	_	_	_
ME.2Ea	SP circumcized	_	_	_	_
ME.4	STD's				
ME.4Aa	**Genital herpes**	_	_	_	_
ME.4Ab	Date primary	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4Ac	# recurrences/yr	_ _	_ _	_ _	_ _
ME.4Ad	Uses acyclovir	_	_	_	_
ME.4Ae	Sexual IC c recur	_	_	_	_
ME.4B	**#x's Gonorrhea**	_	_	_	_
ME.4Ba	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4C	**#x's NGU/CERV**	_	_	_	_
ME.4Ca	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4D	**#x's syphilis**	_	_	_	_
ME.4Da	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4E	**#x's Genital warts**	_	_	_	_
ME.4Ea	Date of dx	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _

ME.4F	**#x's genital ulcers**	_	_	_	_
ME.4Fa	Date of dx	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4G	**#x's vaginitis**	_	_	_	_
ME.4Ga	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4H	**Hepatitis**	_	_	_	_
ME.4Ha	Date of dx	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4I	**Any STD**	_	_	_	_
ME.5	CURRENT MEDICAL HISTORY				
	In past month, have you had:				
ME.5A	Lymphadenopathy	_	_	_	_
ME.5B	Fever >101	_	_	_	_
ME.5C	Night sweats	_	_	_	_
ME.5D	Fatigue	_	_	_	_
ME.5E	Weight loss >10 lbs	_	_	_	_
ME.5F	Anorexia	_	_	_	_
ME.5G	Cough	_	_	_	_
ME.5H	Dyspnea	_	_	_	_
ME.5I	Diarrhea >3 x/d	_	_	_	_
ME.5J	Skin problems	_	_	_	_
ME.5K	Mouth sores	_	_	_	_
ME.5L	Oral thrush	_	_	_	_
ME.5M	Easy bruising	_	_	_	_
ME.5N	Easy bleeding	_	_	_	_
ME.5O	Other	_	_	_	_

INDETERMINATE WESTERN BLOT STUDY

PHYSICAL EXAM (REV092589)

PE.0	Subject Id	____			
PE.0A	Visit Number	1	3	6	9
PE.0B	Visit Date	_____	_____	_____	_____
PE.1	EXAM 0=nl;1=abnl;.=ND;*=unable to do				
PE.1A	Mental status	_	_	_	_
PE.1B	Fundi	_	_	_	_
PE.1C	Oral exam	_	_	_	_
PE.1Ca	Thrush	_	_	_	_
PE.1Cb	Hairy leukoplakia	_	_	_	_
PE.1Cc	Kaposi's	_	_	_	_
PE.1Cd	Other	_	_	_	_
PE.1D	Skin exam	_	_	_	_
PE.1Da	Lesions	_	_	_	_
PE.1E	Abdominal exam	_	_	_	_
PE.1Ea	Liver 0=nl;1=enlarged_____	_	_	_	_
PE.1Eb	Spleen 0=nl;1=enlarged____	_	_	_	_
PE.1F	Pulmonary exam	_	_	_	_
PE.2	NODES				
PE.2A	Ant cerv	_	_	_	_
PE.2B	Post cerv	_	_	_	_
PE.2C	Pre/post auricular	_	_	_	_
PE.2D	Axillary	_	_	_	_
PE.2E	Inguinal	_	_	_	_

INDETERMINATE WESTERN BLOT STUDY

LABORATORY RESULTS (REV891030)

LR.0	Subject Id	_ _ _ _				
LR.0A	Visit #	_	_	_	_	_
LR.0B	Visit Date	_____	_____	_____	_____	_____
LR.1	ELISA 0=NR 1=borderline 2=Repeatedly reactive	_	_	_	_	_
LR.1A	OD ratio to cut-off	_._ _	_._ _	_._ _	_._ _	_._ _
LR.1B	OD value	_._ _	_._ _	_._ _	_._ _	_._ _
LR.1C	ELISA Brand 1=Dupont 2=Epitope 3=other 9=unk	_	_	_	_	_
LR.2A	WESTERN BLOT 1 0=negative; 1=indeterminate 3=positive	_	_	_	_	_
LR.2Aa	WB Brand 1=Dupont 2=Epitope	_	_	_	_	_
LR.2Ab	Bands present:					
LR.2Ab1	p17/18	_	_	_	_	_
LR.2Ab2	p24	_	_	_	_	_
LR.2Ab3	p31	_	_	_	_	_
LR.2Ab4	gp41	_	_	_	_	_
LR.2Ab5	p51	_	_	_	_	_
LR.2Ab6	p55	_	_	_	_	_
LR.2Ab7	p66	_	_	_	_	_
LR.2Ab8	gp120	_	_	_	_	_
LR.2Ab9	gp160	_	_	_	_	_
LR.2Ab10	other/HLA	_	_	_	_	_
	specify	.....	.....	.....	.....	.....

LR.2B	WESTERN BLOT 0=negative 1=indeterminate 2=positive	–	–	–	–	–
LR.2Ba	WB Brand 1=Dupont 2=Epitope	–	–	–	–	–
LR.2Bb	Bands present:					
LR.2Bb1	p17/18	–	–	–	–	–
LR.2Bb2	p24	–	–	–	–	–
LR.2Bb3	p31	–	–	–	–	–
LR.2Bb4	gp41	–	–	–	–	–
LR.2Bb5	p51	–	–	–	–	–
LR.2Bb6	p55	–	–	–	–	–
LR.2Bb7	p66	–	–	–	–	–
LR.2Bb8	gp120	–	–	–	–	–
LR.2Bb9	gp160	–	–	–	–	–
LR.2Bb10	other	–	–	–	–	–
	specify	.....	.....	.....	.....	.....
LR.3	RIPA 0=neg;1=borderline 2=positive		–	–	–	–
LR.3A	Bands present					
LR.3Aa	p24		–	–	–	–
LR.3Ab	p55		–	–	–	–
LR.3Ac	gp120/160		–	–	–	–
LR.4	PCR-CETUS 0=neg;1=borderline; 2=positive		–	–	–	–
LR.5	PCR-UofW 0=neg;1=borderline; 2=positive		–	–	–	–
LR.5	Serum p24 Ag		–	–	–	–

LR.6	T-cell subsets				
LR.6A	%T4	_ _	_ _	_ _	_ _
LR.6Aa	AbsT4	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.6B	%T8	_ _	_ _	_ _	_ _
LR.6Ba	AbsT8	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.6C	T4:T8	_._ _	_._ _	_._ _	_._ _
LR.7	Rheumatoid Fx	_	_	_	_
LR.7A	Titer 1:	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.8	ANA	_	_	_	_
LR.8A	Titer 1:	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.8B	Pattern 1=speckled 2=diffuse 3=homo 4=nucleoler	_	_	_	_
LR.9	RPR	_	_	_	_
LR.10	Other antibodies	_	_	_	_
LR.11	HTLV-1 ELISA 0=neg;1=WR;2=pos	_	_	_	_
LR.12	HTLV-1 western blot 0=nl;1=inde;2=pos	_	_	_	_
LR.12A	Bands present				
LR.12Aa	p15	_	_	_	_
LR.12Ab	p19	_	_	_	_
LR.12Ac	p24	_	_	_	_
LR.12Ad	gp21	_	_	_	_
LR.12Ae	gp46	_	_	_	_
LR.12Af	p40x	_	_	_	_
LR.12Ag	p96	_	_	_	_
LR.12Ah	other	_	_	_	_
	specify	.....	.....	.....	.....

LR.13	FeLV WB 0=neg;1=bordeline; 2=pos	–	–	–	–
LR.14	FIV WB 0=neg;1=borderline; 2=pos	–	–	–	–
LR.15	BLV p24 Agar	–	–	–	–
LR.16	Dupont ENV 9	–	–	–	–
LR.17	HIV cultures				
LR.17A	Plasma	–	–	–	–
LR.17Aa	Day positive	– –	– –	– –	– –
LR.17B	Cells	–	–	–	–
LR.17Ba	Day positive	– –	– –	– –	– –
LR.18	HIVAGEN	–	–	–	–
LR.18A	Bands present				
LR.18Aa	Ip24	–	–	–	–
LR.18Ab	Kp55	–	–	–	–
LR.18Ac	Kp41	–	–	–	–
LR.18Ad	Kp120N	–	–	–	–
LR.18Ae	Kp120CC	–	–	–	–
LR.18Af	Kp66/31	–	–	–	–
LR.18Ag	Ip120	–	–	–	–
LR.19	Hepatitis panel				
LR.19A	HBsAg	–	–	–	–
LR.19B	HBsAb	–	–	–	–
LR.19C	HBcAB	–	–	–	–
LR.19D	PCR-HB rev tran pri	–	–	–	–

APPENDIX B

INDETERMINATE WESTERN BLOT PSYCHOLOGICAL QUESTIONNAIRE

	Variable
Study No.	____
Study Visits: 0=prior to 1st visit; 1=initial; 3=3 mos; 6=6mos; 9=9mos	____
Subject Status: 0=case; 1=control	____
Site:	____

1 = AIDS Prevention Project
2 = Puget Sound Blood Center
3 = Tacoma/Pierce County Blood Center
4 = Dept of Public Health
5 = HMC STD Clinic
6 = Planned Parenthood
7 = UW/HMC Women's Clinic
8 = Methadone Clinic
9 = TB Clinic
10=Immigration
11=private medical doctor
12=Portland Red Cross
13=military
14=insurance
15=other_____

Today's date ____/____/____ (M D Y)

Date notified of HIV test result ____/____ (M Y)

The following questions pertain to your experiences and feelings about your indetermir Western blot result. There are no right answers to these questions. We are interested your thoughts and reactions at the present time.

1. What do you think an indeterminate Western Blot means?

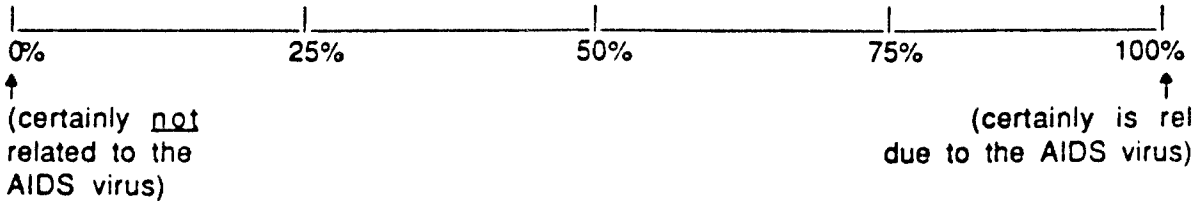
a) The likelihood that my Western Blot is indeterminate due to a limitation of the laboratory or the test is:
[Place an "X" on the scale]

|_____|_____|_____|_____|
0% 25% 50% 75% 100%

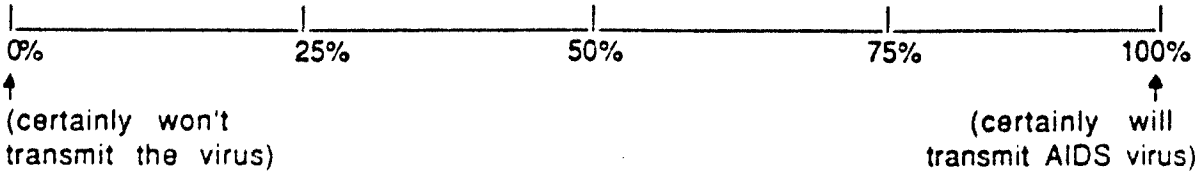
(my indeterminate is not due to a limitation of the lab or test)

(my indeterminate is certainly a limitation of the lab or test)

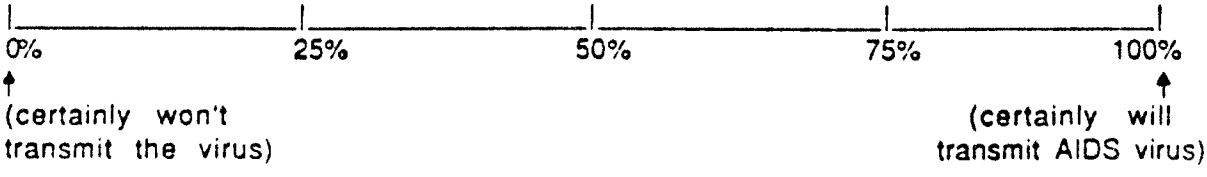
b) The likelihood that my Western Blot is indeterminate because of some abnormality in my blood related to the AIDS virus is:



2. Please indicate what you think the likelihood is that you will transmit the AIDS virus your sexual partner.



3. Please indicate the likelihood of your transmitting the AIDS virus through non-sexual contact to household members or work associates.



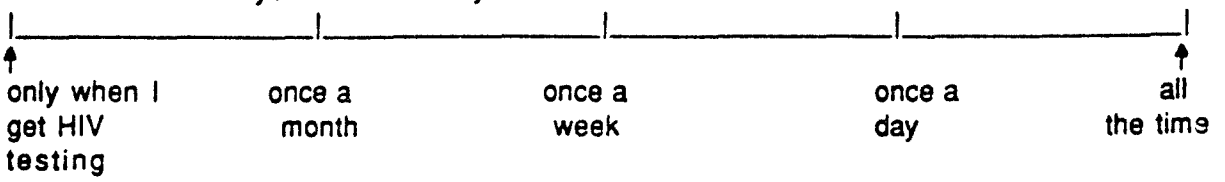
4. Please indicate which of the following you think is most likely to happen to your indeterminate Western blot.
[check one response only]

My Western blot is most likely to:
turn negative_____
remain indeterminate_____
turn positive_____

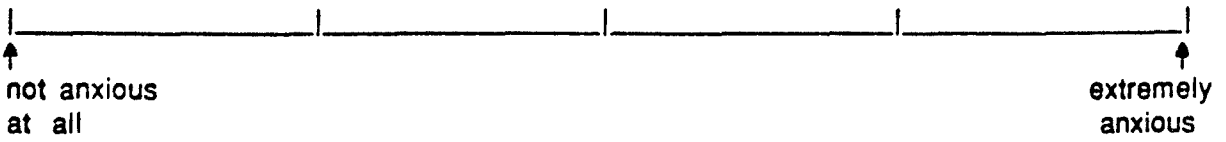
5. If your Western blot does not turn positive over the next six months, will you feel completely reassured that you have not been exposed to the AIDS virus?
[check one response only]

Yes_____
No_____
Uncertain____

6. How often do you think about your indeterminate Western blot?



7. How anxious are you now about your indeterminate Western blot?



Below is a list of complaints that you may have had since being notified of your AIDS test result. Read each item carefully and select one of the descriptions that best describes HOW MUCH DISCOMFORT THAT PROBLEM HAS CAUSED YOU SINCE YOU RECEIVED YOUR AIDS TEST RESULT.

0=Not at all; 1=A little bit; 2=Moderately; 3=Quite a bit; 4=Extremely

A.	There has been a negative effect on my self-esteem.	A._____
B.	Nervousness and shakiness inside.	B._____
C.	Trembling.	C._____
D.	Suddenly scared for no reason.	D._____
E.	Feeling fearful.	E._____
F.	Heart pounding or racing.	F._____
G.	Feeling tense or keyed up.	G._____
H.	Spells of terror or panic.	H._____
I.	Feeling so restless you couldn't sit still.	I._____
J.	The feeling that something bad is going to happen to you.	J._____
K.	Thoughts and images of a frightening nature.	K._____

Below are 13 sets of statements. Please circle the statement in each set which you believe to be most descriptive of yourself. Be sure to read all the statements in each group before making your choice.

A. 0. I do not feel sad.
1. I feel sad or blue.
2. I am blue or sad all the time and I can't snap out of it.
3. I am so sad or unhappy that I can't stand it.

B. 0. I am not particularly pessimistic or discouraged about the future.
1. I feel discouraged about the future.
2. I feel I have nothing to look forward to.
3. I feel that the future is hopeless and that things cannot improve.

C. 0. I do not feel like a failure.
1. I feel I have failed more than the average person.
2. As I look back on my life, all I can see is a lot of failures.
3. I feel I am a complete failure as a person (parent, husband, wife).

D. 0. I am not particularly dissatisfied.
1. I don't enjoy things the way I used to.
2. I don't get satisfaction out of anything anymore.
3. I am dissatisfied with everything.

E. 0. I don't feel particularly guilty.
1. I feel bad or unworthy some of the time.
2. I feel quite guilty.
3. I feel as though I am very bad or worthless.

F. 0. I don't feel disappointed in myself.
1. I am disappointed in myself.
2. I am disgusted with myself.
3. I hate myself.

G. 0. I don't have any thoughts of harming myself.
1. I feel I would be better off dead.
2. I have definite plans about committing suicide.
3. I would kill myself if I had the chance.

H. 0. I have not lost interest in other people.
1. I am less interested in other people than I used to be.
2. I have lost most of my interest in other people and have little feelings for them.
3. I have lost all of my interest in other people and don't care about them at all.

I. 0. I make decisions about as well as ever.
1. I try to put off making decisions.
2. I have great difficulty in making decisions.
3. I can't make any decisions at all any more.

J. 0. I don't feel I look any worse than I used to.
1. I am worried that I am looking old or unattractive.
2. I feel that there are permanent changes in my appearance and they make me look unattractive.
3. I feel that I am ugly or repulsive looking.

K. 0. I can work about as well as before.
1. It takes extra effort to get started at doing something.
2. I have to push myself very hard to do anything.
3. I can't do any work at all.

L. 0. I don't get any more tired than usual.
1. I get tired more easily than I used to.
2. I get tired from doing anything.
3. I get too tired from doing anything.

M. 0. My appetite is no worse than usual.
1. My appetite is not as good as it used to be.
2. My appetite is much worse now.
3. I have no appetite at all any more.

Below you will find a series of 14 life events (A - N) that may have occurred from six months ago to the present. Please indicate (putting a check in the appropriate column) the degree to which you believe the event has caused you distress. If the event did not occur, please check that column ("did not occur")

LIFE EVENT	did not occur	no distress	mild distress	moderate distress	severe distress
A. Change in school situation					
B. Change in work situation					
C. Engagement or marriage					
D. Change in marital status (divorce or separation)					
E. Change in relationship with spouse/ significant other					
F. Pregnancy or birth of a child					
G. Serious family arguments (not including spouse)					
H. Death of family member or friend					
I. Change in residence to a different city/town					
J. Change in financial situation/status					
K. Financial debt					
L. Change in physical health (or injury)					
M. Serious illness or injury of close family member					
N. Divorce or separation of parents					
O. Decision to be tested for HIV (AIDS virus)					
P. Results of HIV test					

Indicate which of the above experiences (A-P) has been the major stressor (problem) in your life: _____

If there has been another experience that is not listed above, please indicate it.

Open-ended questions

1. Can you think of other events in your life that have had as significant an impact on you your feelings or functioning, as your indeterminate Western Blot?
   What were those events?

2. Are there current stressors in your life other than the indeterminate Western blot? [eg., change of job, death in family, loss of friend, change in your health]

3. Have you changed your behavior as a result of the indeterminate Western blot [eg., decrease in frequency of sexual activity, decrease in number of sexual partners, increase in missed days of work, sought counseling, increase or decrease in alcohol or drug use]?

4. In retrospect, what information do you wish you could have obtained about the Western blot, especially when you first learned that yours was indeterminate?

5. Is there anything else about the impact of the indeterminate Western blot on you that we have not covered?

6. How helpful was this interview with the study researcher compared to your other sources of information?

   What were the best features of your interview with us?
   What were the worst features?

FOR PROVIDERS

APPENDIX C

Additional Information about Indeterminate Western Blots

The serum sample submitted from this individual was repeatedly reactive by HIV-1 enzyme-linked immunosorbent assay (EIA) and indeterminate by Western blot. Both clinicians and patients have reported difficulty in understanding the significance of an indeterminate HIV-1 Western blot (IWB).

This uncertainty prompted the University of Washington to conduct a study on the causes, risk of seroconversion, specificity of supplemental tests, and psychological impact of IWB. The study enrolled individuals with IWB from April 1988 through April 1991. Since the study is no longer open for new enrollments, the study investigators have written these brochures for clinicians to summarize the current state of our knowledge and to aid in counseling and evaluating persons with IWB.

How common is an indeterminate Western blot (IWB)?

The prevalence of IWBs depends on the prevalence of HIV infection, other medical conditions, and autoantibodies in the population being tested; inter-lot variability in the amount of HIV-1 antigen on the Western blot strips; and the laboratory's experience in performing and interpreting Western blots. The published prevalence of indeterminate results has ranged from 10% in EIA reactive military recruits to 13% of EIA reactive Minnesota blood donations (1).

What causes indeterminate Western blots?

Indeterminate results occur in HIV infection in the seroconversion window, when only core antibody (e.g., p24) may be detected by Western blot, and late in AIDS when core antibody levels decline (2,3). Autoantibodies may account for cross-reactivity with the Western blot, as in systemic lupus erythematosis and among some healthy individuals. Among women, alloimmunization due to current or past pregnancies may be associated with IWB (unpublished data, Univ of Wash study, 1991). Cross-reactivity with HIV-2 can occur, but cross-reactivity does not occur with HTLV-1 (4,5).

What is the likelihood someone with an IWB is infected with HIV?

The risk of true HIV infection among individuals with IWB is determined by the rate of seroconversion to positive results. This rate was 0-5% in the published blood donor cohorts, 90% among a San Francisco cohort of 20 individuals with p24 antibodies on initial Western blots, and 4% in the University of Washington study (4,6-9). The likelihood that an IWB reflects HIV infection is dependent on HIV risk factors and the time to seroconversion. The highest risk of seroconversion appears to be for those with a p24 band.

What are the potential adverse consequences of an IWB?

We have interviewed over 200 individuals with IWB referred to us from health department clinics, blood banks, prenatal clinics, and private providers. The uncertainty engendered by an indeterminate result, the need for at least 6 months follow-up, and discriminatory policies regarding insurability and immigration have been very distressing to both low- and high-risk individuals in our study population. For example, several persons with IWB referred to us have experienced difficulty in obtaining life or disability insurance, elective surgery, in vitro fertilization, visas for foreign travel, and U.S. citizenship. Although none of these individuals were infected with HIV, they were treated as if they were infected, sometimes for an indefinite period of time.

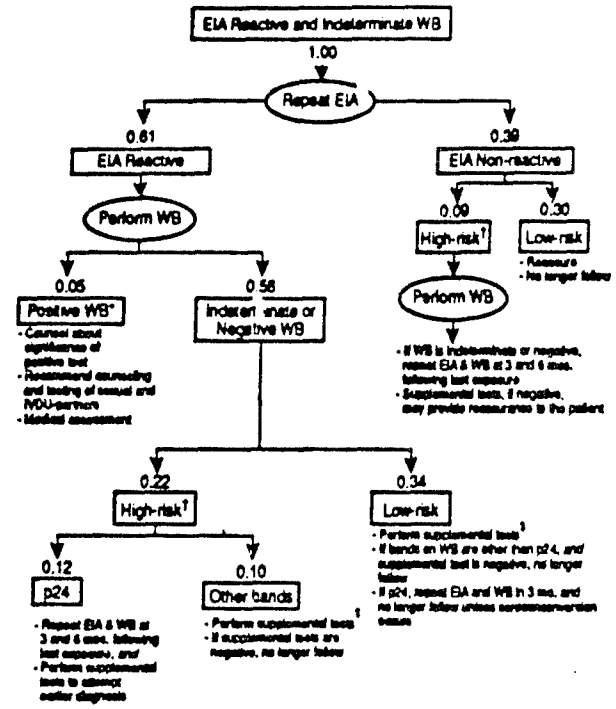
When counseling individuals with IWB, we recommend discussing the following:

- the absence of seroconversion in low-risk individuals
- possible etiologies for the inconclusive result (e.g., cross-reacting antibodies, autoantibodies, and in high-risk persons, possible early seroconversion)
- the need for safe sexual and drug-use practices pending resolution of the test result for high-risk individuals
- recommendations for evaluation, as described below

How should an individual with an IWB be followed?

The CDC recommends that low-risk individuals with an IWB pattern be followed for at least six months; if the Western blot pattern persists as indeterminate or turns negative, then the individual can be reassured that he or she is not infected with HIV (10). Additional diagnostic follow-up is recommended for high-risk persons, including serial testing by Western blot, assessment of immune function, and HIV testing of the person's sexual and needle-sharing partners. Limited data exist about the use of supplemental virologic evaluation of individuals with indeterminate results. Such an evaluation may include HIV-1 culture, recombinant or radioimmunoprecipitation assays, or selective HIV-1 proviral DNA amplification utilizing PCR, if available.

Based upon the University of Washington study of IWB, a sequential process for evaluation was proposed that would provide more rapid determination of the HIV status of an individual with an IWB (9) (see next page):



KEY POINTS

- Risk assessment is very important
- Repeat EIA and WB after 1 month
- Follow high-risk persons with repeat EIA & WB at 3 and 6 months
- No need to follow low-risk persons with bands other than p24 or blots that turn negative

Legend:

* WB = Western blot. The numbers on the algorithm indicate the proportion of cases in the University of Washington study who were in different groups, based on their ELISA and WB results at the first study visit (N = 89 cases).

† High-risk includes recipients of blood products between 1978 and 1985, intravenous drug users (IVDU), homosexual and bisexual men, and sexual partners of IVDU and homosexual and bisexual men.

‡ In most cases supplemental tests will be optional and an individual's HIV status will be clarified by serologic follow-up over three-six months. If available, supplemental tests to consider performing are HIV-1 culture, PCR, or a recombinant envelope antigen assay.

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- For additional information, please call the U. W. Center for AIDS Research at (206) 720-4298 -

Provided by the University of Washington Department of Laboratory Medicine, Division of Virology and the Indeterminate Western Blot Study

AN "INDETERMINATE" HIV-1 WESTERN BLOT: WHAT DOES IT MEAN?

Q: What are the HIV-1 (AIDS) antibody tests?

A: To screen for HIV-1 antibodies, the laboratory first performs an ELISA or EIA test. This is a very accurate test, but occasionally it picks up antibodies that are not caused by HIV and which cause false-positive EIAs. False-positive EIAs occur because the immune system produces countless antibodies during the process of fighting off diseases, which can "cross-react" with the EIA.

To determine whether a positive EIA is a true or false-positive, a confirmatory Western blot is performed which detects antibodies to individual characteristic proteins that make up the HIV virus. The Western blot is called positive if several antibodies are present, negative if no antibodies are present, and indeterminate if bands representing antibodies are present that don't meet the criteria for a positive Western blot. The Western blot is also a very accurate test, but occasionally it too can detect "cross-reacting" antibodies.

Q: How common is an indeterminate Western blot (abbreviated as IWB)?

A: Among low-risk persons who are reactive by HIV-1 EIA, 10% will be indeterminate by Western blot.

Q: What causes indeterminate Western blots?

A: It appears that many indeterminate Western blots are due to cross-reacting antibodies which may be found in some healthy individuals as well as others with medical conditions such as lupus or rheumatoid arthritis. While these conditions may cause an IWB, it is important to note that an IWB itself does not indicate or diagnose other conditions. Women with previous pregnancies may also have cross-reacting antibodies present.

Occasionally an IWB can be seen very early in HIV infection in the first few months after an individual has been infected with the virus. In the University of Washington IWB study, we found that approximately 4% of the individuals referred to us with IWB were infected with HIV but hadn't yet formed all the antibodies initially needed to call the test positive.

Q: What is the likelihood someone with an IWB is infected with HIV?

A: It depends on whether that individual recently had high-risk sexual contacts or intravenous drug use. In a recent University of Washington study, individuals with an IWB and no reported risk factors for HIV infection were not infected with HIV.

Q: How can someone find out if the IWB is due to HIV infection?

A: The current recommendation from the Centers for Disease Control is to repeat the EIA and Western blot several times over six months to see whether the Western blot becomes positive. If the IWB stays indeterminate or turns negative, the person is not HIV-infected.

Q: How will an IWB affect my eligibility to donate blood or obtain life insurance?

A: Currently the Food and Drug Administration (FDA) requires that blood banks not allow blood donors with IWB to donate blood because of the small chance that they could be recently infected with HIV. The FDA is understandably taking a cautious approach to avoid infecting any transfusion recipients. You can find out whether the blood center policy about donors with IWB has changed by calling your blood center. Occasionally individuals have been temporarily deferred from obtaining life insurance because of an IWB until follow-up tests indicate they are not HIV-infected.

Q: What should I do now?

A: Discuss your concerns about the IWB with your health care provider, including a thorough discussion about possible HIV risk factors. It is very important to be fully honest with your health care provider about your sexual and drug-related behaviors so that he or she can decide how long to follow you and whether to perform additional tests. It is advisable to observe safe sexual practices (eg., using condoms and avoiding anal intercourse) and to avoid sharing needles, in the case of persons who inject drugs. For both low- and high-risk persons, some anxiety about the test result is understandable. We hope the information in this brochure, combined with discussions with your health care provider, will help reassure you.

Provided by
University of Washington
Dept. of Laboratory Medicine,
Division of Virology and the
Indeterminate Western Blot Study

APPENDIX D

DUPLICATE

This set of proofs shows all printer's marks or queries. Author or Editor will please indicate all corrections on this set.

AUTHOR - Please see query on manuscript page 21, 001, 24.

Indeterminate Human Immunodeficiency Virus Type 1 Western Blots: Seroconversion Risk, Specificity of Supplemental Tests, and an Algorithm for Evaluation

Connie L. Celum, Robert Coombs, William Lafferty, Thomas S. Inui, Pamela H. Louie, Carol A. Gates, Bruce J. McCreedy, Richard Egan,* Thomas Grove, Steve Alexander, Thomas Koepsell, Noel Weiss, Lloyd Fisher, Lawrence Corey, and King K. Holmes

Departments of Medicine, Laboratory Medicine, Epidemiology, and Biostatistics, University of Washington, and HIV/AIDS Epidemiology Office, Washington Department of Health and Social Services, Seattle; Roche Diagnostic Research Laboratories and Cetus Corporation, Emeryville, and SmithKline Beecham Clinical Laboratories, Van Nuys, California; Center for Molecular Biology, Roche Biomedical Laboratories, Research Triangle Park, North Carolina; Dupont Glasgow Research Laboratory, Glasgow, Delaware; Cambridge Biotech, Rockville, Maryland

The human immunodeficiency virus type 1 (HIV-1) Western blot is indeterminate in 10%–20% of sera reactive by EIA. Eighty-nine individuals with prior repeatedly reactive EIA and indeterminate Western blots were followed prospectively to study the risk of seroconversion and specificity of supplemental tests. Four high-risk cases seroconverted within 10 months after enrollment (seroconversion risk, 4.5%, 95% confidence interval, 1.2%–11.1%). Among cases with p24 bands initially, 4 (18.2%) of 22 high-risk individuals seroconverted compared with 0 of 33 low-risk cases ($P = .03$). Specificities of HIV-1 culture, serum p24 antigen, polymerase chain reaction, and recombinant ENV 9 EIA were 100%, 100%, 98.6%, and 94.4%, respectively. An expedited evaluation protocol is proposed. Low-risk individuals with nonreactive EIAs upon repeat testing do not need further follow-up; high-risk individuals should be followed serologically for at least 6 months, especially those with p24 bands on Western blot.

The first laboratory step in human immunodeficiency virus type 1 (HIV-1) antibody detection is the EIA which has a reported sensitivity and specificity of >99% [1–5]. Specimens that are repeatedly reactive by HIV-1 EIA are confirmed by a more specific supplemental test, which is usually the Western blot. The Western blot detects antibodies to specific denatured HIV-1 proteins, such as core (p17, p24, and p55), polymerase (p31, p51, p66), and envelope (gp41, gp120, gp160) proteins [6–8]. The Western blot has a reported specificity of 97.8% [5]. About 10%–20% of sera that are repeatedly reactive by HIV-1 EIA are interpreted as indeterminate by Western blot [8–11].

Indeterminate HIV-1 Western blots (IWBs) may be due to antibody production against viral core antigens early in HIV-1 infection [12–14], loss of core antibodies late in HIV-1 infection [15, 16], cross-reactive antibody to HIV-2 [17], or cross-reactive antibody due to autoantibodies or alloimmunization [18–21]. Because an IWB may represent recent HIV-1 infection and incomplete antibody production, the Centers for Disease Control (CDC) recommends that all individuals with IWBs be retested over 6 months. The CDC recommends that a low-risk individual be considered HIV-negative if the Western blot is still indeterminate or becomes negative after 6 months. Longer follow-up, HIV-1 testing of sex and drug-using partners, and additional immunologic and virologic evaluation are recommended for high-risk individuals with IWBs [8].

Individuals with IWBs are currently excluded from blood donations and have had difficulty obtaining life and disability insurance, US immigration status, and visas for foreign travel. Concern about possible HIV-1 infection among those with IWBs has resulted in uncertainty about appropriate procedures for notification, counseling, and evaluation. A clearer estimation of the risk of seroconversion among individuals with IWBs is needed. Accurate identification of HIV-1 infection among individuals with IWBs may be possible by use of supplemental HIV-1 tests, including HIV-1 culture, serum p24 antigen assay, polymerase chain reaction,

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Informed consent was obtained from patients or their guardians, and the study protocol was approved by the University of Washington institutional review board.

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Reprints or correspondence: Dr. Connie Celum, Division of Infectious Diseases, Harborview Medical Center ZA-09, 325 9th Ave., Seattle, WA 98104.

* Present address, Syva Corporation, Palo Alto, California.

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radioimmunoprecipitation assay, and recombinant HIV-1 antigen assays, but the sensitivity and specificity of these supplemental tests in individuals with IWBs is not known.

This study was designed to assess the risk of seroconversion and the specificity of supplemental HIV-1 tests in a prospective cohort of both low- and high-risk individuals referred because of repeatedly reactive EIAs and IWBs.

Methods

Study population and design. A prospective cohort study with 6–9 months of follow-up was initiated at the University of Washington in March 1988. The cohort included men and women 16–70 years of age with one or more repeatedly reactive EIAs and an IWB, who were referred from testing sites in Washington and Oregon. We accepted the HIV-1 Western blot interpretive criteria of the referral laboratory for Western blots done on subjects before study enrollment. Individuals with a prior diagnosis of HIV seropositivity or AIDS were excluded from the study. Subjects were interviewed about HIV risks [22] and general medical history and were examined.

Laboratory studies. The three reference laboratories doing EIAs and Western blots for the study subscribe to the College of American Pathologists proficiency panel for HIV-1 antibody testing. Dupont (Biotech Research Laboratory, Rockville, MD) EIA and Epitope (Beaverton, OR) and Dupont Western blots were used. The CDC interpretive criteria were used for both Epitope and Dupont Western blots: a Western blot was considered positive if antibodies were present to two of the following HIV-1 viral proteins: p24, gp41, and gp120/gp160 [8]. Western blots without any bands were considered negative, and blots with bands not meeting the criteria for a positive blot were interpreted as indeterminate.

Cases were followed prospectively with repeat HIV-1 EIAs and Western blots every 3 months. The diagnosis of HIV-1 infection was based on seroconversion (a positive EIA and Western blot) or on isolation of HIV-1 in culture from peripheral blood mononuclear cells (PBMCs). Positive Western blots were repeated to rule out laboratory error.

Specificity of supplemental HIV-1 tests. Determination of the specificity of supplemental tests was based on test results from the individuals who did not develop a positive HIV-1 culture or positive Western blot during ≥6 months of follow-up. Supplemental tests were done on sera and cells obtained from subjects at the first study visit and on samples from 81 HIV-1 EIA–negative controls recruited from the same HIV testing sites.

HIV-1 cultures and serum p24 antigen. Cell-free plasma and PBMCs were cultured for HIV-1 as previously described [23, 24]. Culture supernatants were sampled for HIV-1 p24 antigen with the antigen-capture EIA following the manufacturer's protocol (Abbott Laboratory, Chicago, IL) every 3 days for 1 month. Serum p24 antigen assays were done by the same antigen-capture EIA method [25–27]. Positive serum samples were tested in a confirmatory antibody-neutralization assay.

Polymerase chain reaction (PCR). PCR was done by Cetus (Emeryville, CA) and Roche Biomedical Laboratories (Research Triangle Park, NC) using the SK38/39 and SK101/145 primer pairs for the HIV-1 *gag* gene [28, 29]. Cell lysates were obtained from cryopreserved PBMCs, and amplification competency of specimens was checked by amplification of a conserved region within the histocompatibility locus antigen–DQα locus with primer pair GH26/27 [30]. HIV-1 DNA amplification was done as described by Kellogg and Kwok [28]. Each specimen was run in duplicate for both primer sets. HIV-1 proviral sequences were considered present if both primer pairs were positive in duplicate, indeterminate if only one of the duplicate reactions was positive for one or both primer pairs, and not present if neither primer pair resulted in a positive signal.

Serologic assays. Two serologic EIAs of recombinant HIV-1 antigens were done by their manufacturers: HIVAGEN (SmithKline Beecham Clinical Laboratories, Van Nuys, CA) and ENV 9 (Dupont Glasgow Research laboratory, Glasgow, DE). The HIVAGEN panel comprised five recombinant HIV-1 antigens produced in *Escherichia coli:* Ip24 represents the entire sequence of p24, Kp55 the complete sequence of p55, Kp66/31 the complete reverse transcriptase genome and 40% of endonuclease, Kp41 40% of the amino-terminus of gp41, and Igp120 98% of gp120 [31]. A HIVAGEN result that showed Ip24, Kp55, or Kp66/31 and either Kp41 or Igp120 was considered positive, and any other pattern of reactivity was considered indeterminate. ENV 9 used a single HIV-1 envelope peptide (the carboxy-terminus of gp120 and half of the gp41 sequence) produced in *E. coli* [32].

Radioimmunoprecipitation assays (RIPAs) were done by Biotech using HIV-1–infected H-9 cells labeled for 8 h with [^{35}S]methionine [33]. Samples reactive with the envelope glycoproteins gp120 and gp160 were considered positive.

Statistical methods. Demographic and HIV risk factors were compared using χ^2 analysis and Fisher's exact test for categorical data and Student's *t* test for continuous data. Logistic regression was used to compare the proportion of cases with reactive versus nonreactive HIV-1 EIA at visit one with respect to the proportion with past high-risk sex partners while controlling for time between initial HIV tests and study enrollment and for the number of HIV tests before study enrollment. The Mann-Whitney test was used for comparing continuous distributions when the assumption of a normal distribution was not appropriate. Specificity of the supplemental HIV-1 tests was analyzed by comparing results of the supplemental tests with the 6-month Western blot result and isolation of HIV-1 by culture as the reference standards. Ninety-five percent confidence intervals (CI) for the seroconversion risk were calculated using exact binomial methods.

Results

Of 147 individuals referred and enrolled in the study as of May 1990, 89 were followed for ≥6 months and were included in this analysis. Five subjects moved or were lost to follow-up and were not included in the analysis, and the remaining 53 have been followed for <6 months. Subjects were referred primarily from blood banks (49%) and from Department of Public Health clinics (29%). The reasons cited for HIV-1 testing were routine HIV-1 screening for

blood donors, military recruits, or life insurance or immigration applicants (58%), concern over past sexual exposures (27%), current or past intravenous drug use (2%), pregnancy (5%), needlesticks in health care workers (2%), and other reasons such as prior blood-product transfusion or hemophilia (6%).

The subjects were divided into three groups for analysis based on the Dupont HIV-1 EIA and Western blot results on samples obtained at the first study visit. Those in group 1 were four individuals who seroconverted, three of whom seroconverted by the first visit and one who seroconverted 10 months after study enrollment. Group 2 comprised 50 who were still repeatedly reactive by Dupont HIV-1 EIA, with an R-value (ratio of sample to cutoff) ≥0.8 at the first study visit. Group 3 comprised 35 who were no longer reactive by Dupont HIV-1 EIA, with an R-value <0.8 at the first study visit. The seroconversion risk was 4 of 89 (4.5%; 95% CI, 1.2%–11.1%).

The demographics of the three groups were similar except for marital status, with group 3 containing the highest proportion of married subjects (table 1). The proportion of subjects reporting a high-risk sex partner since 1978 was significantly higher for groups 1 and 2 (100% and 34%, respectively) than for group 3 (14%) ($P < .001$, χ^2 analysis). The proportion of cases reporting past bisexual or homosexual male partners was significantly higher for group 1 (75%) than for group 2 (8%) or group 3 (9%) ($P < .001$ for both comparisons). The median time from initial IWB to study enrollment was shortest for group 1 (1 month; range, 0.5–3), intermediate for group 2 (2.5 months; range, 0.5–36), and longest for group 3 (13 months; range, 0.5–51) ($P < .01$ for group 1 vs. group 3; $P < .05$ for group 1 vs. group 2; and $P = .003$ for group 2 vs. group 3). When the proportion of cases with a high-risk sex partner since 1978 was compared between groups 2 and 3 while adjusting for the time between initial IWB and study enrollment and for the number of HIV-1 EIAs before study enrollment, the difference in high-risk sex partners approached statistical significance ($P = .06$).

Characteristics of the four individuals who seroconverted. Case 1 was a bisexual man with a history of prostitution and intravenous drug use before HIV-1 testing in 1988. He reported symptoms of an acute viral-like syndrome in the month between the IWB (p24 antibody only) and the positive Western blot. Case 2 was a woman with a history of autoimmune disease who had unprotected sexual exposure with an HIV-seropositive bisexual partner. After her initial IWB with a p24 band only, she seroconverted by Dupont blot 2 weeks later and by Epitope blot 4 weeks later. Case 3 was a homosexual man with a viral-like syndrome who had p24 and weak gp160 bands (interpreted by the referring laboratory as indeterminate by the FDA/Dupont criteria) and 3 months later had antibodies against all viral proteins on Western blot. Case 4 was a homosexual man with a persistent p24 band and intermittent p66 band until he seroconverted in the tenth month of follow-up. He reported ongoing high-risk sexual behavior during the study period.

Seroconversion was seen only among individuals with p24 bands on their initial Western blots. The risk of seroconversion among individuals with p24 bands was 4 of 55 (7.3%; 95% CI, 2.0%–17.6%). The risk of seroconversion was 4 (18.2%) of 22 among high-risk individuals with p24 bands and 0 of 23 among low-risk persons with p24 bands ($P = .03$ by Fisher's exact test). The median R-value for the seroconverters was 3.6 (range, 3.3–8.5) at the first study visit.

Estimation of the sensitivity of the supplemental HIV-1 tests is not reliable, given the low number of seroconverters. Case 1 had a positive ENV 9 assay and RIPA at the time the initial Western blot showed a p24 band only. Lymphocytes were not available from that visit. When the Western blot became positive 1 month later at his first study visit, his HIV-1 PBMC culture and HIVAGEN assay were positive, but plasma culture, PCR, and serum p24 antigen assay were negative; repeat PCR was positive 3 months after seroconversion. Case 2 had negative serum p24 antigen assay and HIV-1 PBMC and plasma cultures and indeterminate HIVAGEN EIA but a positive PCR, ENV 9 EIA, and RIPA at the initial study visit when the Dupont Western blot detected p17, p24, gp41, and gp120/160 antibodies (2 weeks after the initial Western blot had p24 antibody only). Case 3 was positive on all supplemental tests (HIV-1 PBMC and plasma culture, serum p24 antigen assay, PCR, and ENV 9 and HIVAGEN EIAs) at the first study visit when the Western blot had antibodies against all viral proteins. Specimens were not available from his initial testing 3 months earlier when the Western blot detected antibodies to p24 and gp160. Case 4 had negative HIV-1 PBMC and plasma cultures, PCR, ENV 9 EIA, and RIPA and indeterminate HIVAGEN EIA (Ip24, Kp55) at his initial study visit when the Western blot showed a p24 band only. He seroconverted after 10 months of follow-up, with a history of high-risk behavior intermittently during the 10 months, and refused repeat supplemental testing.

Western blot and supplemental test results in individuals who did not seroconvert. The median R-value of the Dupont HIV-1 EIA was 2.2 (range, 0.9–4.7) among group 2 subjects at the first study visit compared with 0.2 (range, 0.06–0.7) among group 3 subjects. Forty-two group 2 subjects (84%) had repeatedly reactive EIAs at all study visits and 8 (16%) had one or more nonreactive EIAs at follow-up visits. Conversely, 29 group 3 subjects (82.9%) were nonreactive by EIA at all study visits and 6 (17.1%) were again repeatedly reactive at one or more study visits.

There was 70% agreement between Epitope and Dupont blots among the nonseroconverters; 53 cases were indeterminate by both Epitope and Dupont, 24 were indeterminate by Dupont alone, 1 was indeterminate by Epitope alone, and 7 were negative by both Epitope and Dupont (table 2). Among

Table 1. Characteristics of subjects with indeterminate human immunodeficiency virus type 1 (HIV-1) Western blots (IWBs): comparisons by HIV-1 EIA reactivity at first study visit.

	Group 1	Group 2	Group 3	*P*
No.	4	50	35	
Age, years, median (range)	45.5 (22–58)	35 (16–68)	42 (18–70)	NS
No. male (%)	3 (75)	18 (36)	18 (51.4)	NS
No. white (%)	4 (100)	42 (84)	33 (94.3)	NS
Marital status, no. (%)				
Never married	0	20 (40)	8 (22.9)	.002
Married	0	20 (40)	19 (54.3)	
Divorced/separated	4 (100)	7 (14)	8 (22.9)	
Education, years,				
median (range)	14 (13–22)	14 (4–20)	14.5 (9–20)	NS
Annual family income				
>$20,000, no. (%)	2 (50)	22 (46.8)	24 (70.6)	NS
Past sexually transmitted				
diseases*, no. (%)	3 (75)	15 (30)	11 (31.4)	NS
High-risk sex partner				
since 1978, no. (%)	4 (100)	17 (34)	5 (14.3)	<.001
Median (range) no. of				
sex partners past year	4.5 (0–300)	1 (0–50)	1 (0–3)	NS
Sexual preference, no. (%)				
Heterosexual	1 (25)	45 (91.8)	31 (88.6)	<.001
Bisexual	1 (25)	3 (6.1)	2 (5.7)	
Homosexual	2 (50)	1 (2)	1 (2.9)	
Never sexually active	0	0	1 (2.9)	
History of prostitution,				
no. (%)	2 (50)	3 (6)	0	<.001
Intravenous drug use,				
no. (%)	1 (25)	4 (8)	2 (5.7)	NS
Blood product transfusion				
1978–1985, no. (%)	0	3 (6)	3 (8.6)	NS
Time between initial IWB and				
first study visit months,				
median (range)	1.0 (0.5–3)	2.5 (0.5–36)	13 (0.5–51)	<.05
No. of EIAs before study				
enrollment, median (range)	1.0 (1–1)	1 (1–5)	1 (1–5)	NS

NOTE. Subjects were referred because of past repeatedly reactive HIV-1 EIA and new or more IWBs before first study visit. Group 1, individuals who seroconverted during study period (1–10 months); groups 2 and 3, repeatedly reactive and non-reactive, respectively, by HIV-1 EIA (Dupont) at first study visit. *P* values for categorical data were derived from the summary 3×4 χ^2 statistic for groups 1–3. Those for continuous data were obtained from Mann-Whitney tests and represent comparisons between groups 1 and 2, groups 1 and 3, and groups 2 and 3. NS (nonsignificant), $P > .05$.

* Genital herpes, gonorrhea, chlamydial infection, genital warts, genital ulcerations, and hepatitis B.

the 50 group 2 subjects, antibody to p24 was detected by both Dupont and Epitope blots in 11 (22%), by Dupont blot only in 18 (36%), and by Epitope blot only in 1 (2%) at the first study visit ($P < .01$ for comparison between Dupont and Epitope by McNemar's test). Of the 35 group 3 subjects, 12 (34%) had p24 antibody detected by both Dupont and Epitope blots, 10 (29%) had p24 antibody detected only by Dupont blot, and none had p24 antibody detected by Epitope blot only ($P < .01$).

The specificity of supplemental tests done at the initial study visit was estimated in the 85 nonseroconverters who had negative or IWBs after ≥6 months of follow-up (table 3). All 84 HIV-1 cultures were negative in the nonseroconverters. The PCR assay was negative in all 20 EIA-negative controls (data not shown) and 68 (98.6%) of 69 group 2 and 3 subjects who did not seroconvert. One high-risk individual was initially positive by PCR but negative on repeat PCR testing of the same specimen by two different laboratories. During an additional 9 months of follow-up he remained negative for HIV-1 by Western blot, culture, and four serial PCR assays.

ENV 9 EIA was done for 72 nonseroconverters, 4 of whom had borderline reactivity (R-values of 1.1–1.4). Specificity of ENV 9 was 94.4% in the subjects and 100% in 39 EIA-negative controls (data not shown).

Serum p24 antigen testing was done for 64 nonseroconverters; one was borderline reactive but not neutralizable with anti-p24 antibody, resulting in a specificity of 100%.

HIV-1 RIPA was done for 63 nonseroconverters, of whom 50 were negative (79.4%) and 13 were indeterminate

Table 2. Results of human immunodeficiency virus type 1 Western blots at first study visit after enrollment.

	Group 1	Group 2	Group 3
No.	4	50	35
No. negative (%)	0	0	7 (20)*
No. indeterminate (%)	1 (25)†	50 (100)	28 (80)
Epitope only	0	0	1
Dupont only	0	14	10
Epitope and Dupont	1	36	17
No. positive‡ (%)	3 (75)	0	0

NOTE. Subjects were referred because of past repeatedly reactive human immunodeficiency virus type-1 (HIV-1) EIA and one or more indeterminate Western blots before first study visit. Group 1, individuals who seroconverted during study period (1–10 months); groups 2 and 3, repeatedly reactive and nonreactive, respectively, by HIV-1 EIA (Dupont) at first study visit.

* Negative by both Epitope and Dupont blot. Difference in proportion of groups 2 and 3 who had negative versus indeterminate Western blots was significant ($P < .01$).

† Three of four seroconverters had positive Dupont Western blot at first study visit; all three had had p24 band on initial blot. The fourth seroconverted 10 months after initial Western blot showed p24 band only with ongoing risk behavior during study period.

‡ Western blots were interpreted as positive using Centers for Disease Control interpretative criteria if at least two of the following anti-HIV antibodies were present: p24, gp41, gp120/160.

(20.6%). The specificity of RIPA was 79.4% if the indeterminate RIPAs were considered false-positives or 100% if the indeterminate results were excluded.

HIVAGEN EIA was done for 81 nonseroconverters and 63 EIA-negative controls. Sixty-one (75%) of the 81 nonseroconverters were indeterminate and 1 (1%) was positive, and 13 (21%) of the EIA-negative controls were indeterminate by HIVAGEN ($P < .001$). Of the 61 subjects with indeterminate HIVAGEN results, 36% and 72% had reactivity against the Ip24 and Kp55 antigens, respectively, confirming the *gag* reactivity on Western blot. The specificity of the envelope antigens was 100% for Igp120 and 98.8% for Kp41 among the cases.

In summary, excluding indeterminate RIPA and HIVAGEN EIA results, false-positive PCR ($n = 1$) or ENV 9 ($n = 4$) or HIVAGEN ($n = 1$) EIA results were obtained from six subjects, none of whom was positive on more than one supplemental test.

Discussion

The long-term outcome of persons identified as being repeatedly reactive by screening EIA and indeterminate by Western blot for HIV-1 is not well characterized. A more rapid determination of HIV-1 infection among such persons through delineation of epidemiologic and serologic characteristics would benefit both patients and clinicians. In this cohort study of 89 adults referred because of prior reactive HIV-1 EIAs and IWBs, we found HIV-1 infection in only 4 (12.5%) of 32 high-risk cases and 0 of 57 low-risk cases. Of

Table 3. Specificity of supplemental human immunodeficiency virus type 1 (HIV-1) tests in 85 subjects followed ≥6 months who did not develop positive Western blots.

	Group 2 ($n = 50$)		Group 3 ($n = 35$)	
	High risk ($n = 20$)	Low risk ($n = 30$)	High risk ($n = 8$)	Low risk ($n = 27$)
HIV-1 culture negative	20/20	30/30	8/8	26/26
Polymerase chain reaction				
Negative	17/17	26/26	5/6	20/20
Indeterminate	—	—	1/6*	—
Serum p24 antigen negative	17/17	23/23	7/7	17/17
ENV 9 EIA				
Negative	17/19	27/27	4/5	20/21
Low positive†	2/19	—	1/5	1/21
HIVAGEN EIA				
Negative	2/17	1/30	2/8	14/26
Indeterminate	15/17	28/30	6/8	12/26
Positive	—	1/30	—	—
Radioimmunoprecipitation				
Negative	15/18	19/24	5/7	11/14
Indeterminate	3/18	5/24	2/7	3/14

NOTE. Data are no. tests with specified result/no. done. Subjects were referred because of past repeatedly reactive HIV-1 EIA and one or more indeterminate Western blots before first study visit. Group 1, individuals who seroconverted during study period (1–10 months); groups 2 and 3, repeatedly reactive and nonreactive, respectively, by HIV-1 EIA (Dupont) at first study visit.

* Subsequent testing did not confirm initial positive result.

† The four with low-positive results had borderline [illegible] OD values of [illegible]

the 32 high-risk cases, 22 had a p24 band initially, of whom four seroconverted (18%; 95% CI, 5.2%–40.3%); none of the 10 high-risk cases with other bands seroconverted (95% CI, 0–30.9%).

The low risk of seroconversion (4.5%) in our sample population was comparable to that of earlier published studies of blood donors with repeatedly reactive EIAs and IWBs, which reported seroconversion rates of 3%–5% [19–21]. As in our study, the seroconverters in the earlier blood donor cohorts had p24 antibodies on initial Western blot and admitted to HIV risk behaviors. A recent study by Jackson et al. [34] of 99 Minnesota blood donors with indeterminate HIV-1 blots found no evidence of HIV-1 or HIV-2 infection.

During the interval between the first repeatedly reactive EIA and IWB result until enrollment into our study, 39% of cases became nonreactive by EIA, 7 (8%) of whom also were nonreactive by both Epitope and Dupont Western blot. The loss of reactivity on EIA was related to the duration of time between initial testing and the first study visit and the number of prior EIAs done before study enrollment. One explanation for this finding is that these were blood donors who had been tested with earlier generations of less-specific HIV-1 EIAs and Western blots.

An IWB was more persistent than a positive EIA among the nonseroconverters: 79% of the group 3 subjects (no longer EIA-reactive at visit one) still had IWBs. We noted considerable discordance between the proportion of group 2 and group 3 cases who were indeterminate by Dupont and Epitope blots, with the Dupont assay frequently giving indeterminate results on specimens that were negative by Epitope blot. This variability between manufacturers of commercial kits as well as different lots of antigen by the same manufacturer has also been noted by other investigators [35].

Based on our study and the findings of Courouce [36], a nonreactive EIA on a follow-up sample in a low-risk individual with an IWB has a high predictive value for lack of HIV-1 infection, and those individuals do not need further follow-up. Of the 35 group 3 cases, 27 had no risk factors for HIV-1 infection, representing 30% of the total study population who would require no further follow-up by this approach. The remaining 62 subjects (70%) still require additional evaluation based on their risk history or persistent EIA reactivity. Supplemental assays that might more quickly identify or exclude HIV infection would be desirable in this large group.

The low number of seroconverters in our study precluded estimation of the sensitivity of supplemental tests and, therefore, the predictive value of a negative test. Nevertheless, the specificities of HIV-1 culture, PCR, ENV 9 EIA, and serum p24 antigen assay were 100%, 98.6%, 94.4%, and 100%, respectively among the 65 nonseroconverters. We found that HIV-1 culture, PCR, and a recombinant envelope assay were the three most useful supplemental assays. Although HIV-1 culture and PCR have excellent specificity and sensitivity in many laboratories and are reported to be useful in diagnosing the presence or absence of HIV-1 infection [37–40], they are not widely available, currently are technically difficult, and have not been extensively evaluated for sensitivity in this specific context of recently infected individuals with IWBs who have not yet seroconverted.

ENV 9 EIA had a specificity of 94.4% overall, and it or other recombinant envelope assays might be useful as a supplemental test for IWB sera. Prior studies of other recombinant assays, such as CBre3 (Cambridge Biosciences, Boston), have shown high sensitivity in seroconverter panels [41] and excellent negative predictive value in IWBs [42]. The high prevalence of indeterminate recombinant HIVAGEN EIA results in our study population reflected reactivity to one or more *gag* epitopes. The specificity of HIVAGEN recombinant envelope proteins was comparable to that of ENV 9, but the additional core and polymerase proteins did not help to resolve the IWB patterns.

Although the US Army and other investigators have found RIPA to be a sensitive assay compared with Western blot for detecting antibody to HIV-1 envelope glycoproteins during the course of seroconversion [42], we found weak reactivity to p55 or gp120 in 13 (29%) of the 65 nonseroconverters tested. In addition, RIPA is a labor-intensive test that requires radiolabeled lysate and is not practical for routine clinical use.

The p24 antigen assay was 100% specific but detected only one of four seroconverters in our series and was negative in 24 seroconverters before a diagnostic Western blot in another study [38]. A study of p24 antigen screening among male blood donors in the United States found the specificity of p24 antigen to be 100% but the sensitivity only 11.4% [43].

Because the interval from first IWB until study enrollment varied in our cohort, the duration of this interval represents a possible confounder, which we attempted to control for in our analyses. After adjusting for the time and number of prior HIV-1 tests between the initial IWB and study enrollment, the higher proportion of group 2 cases compared to group 3 cases with high-risk sex partners since 1978 approached statistical significance ($P = .06$). This suggests that a factor associated with high-risk sexual contact may account for persistent EIA reactivity and IWBs. This provocative finding may reflect sampling bias or inadequate controlling for confounding but warrants further investigation.

On the basis of our study results, we propose the following algorithm for evaluating individuals with IWBs (figure 1). The first step is to reevaluate the individual's risk behaviors for possible exposure to HIV-1 and to repeat the EIA. Risk assessment, however, will not always accurately identify individuals with risk behaviors [44]; therefore, our recommendations incorporate reported history of risk behavior, persistence of the EIA reactivity, and the presence or absence of p24 antibodies on Western blot. The proportion of individ-

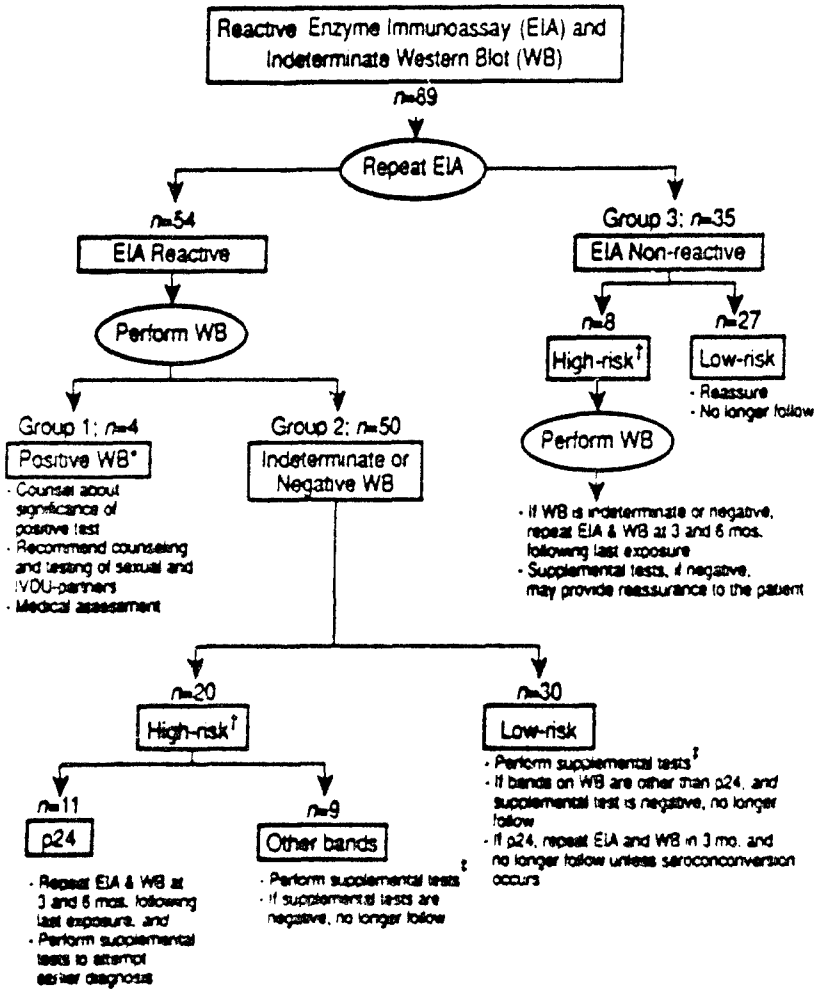

Figure 1. Algorithm for evaluation of individuals with indeterminate human immunodeficiency virus type 1 (HIV-1) Western blots (WBs). * A positive WB was defined as the presence of at least two of the following anti-HIV-1 antibodies: p24, gp41, or gp120/160 (Centers for Disease Control criteria). † High-risk individuals should be followed for at least 6 months after the last exposure (longer if they continue to engage in high-risk behavior). ‡ Supplemental tests include HIV-1 culture, polymerase chain reaction, or a recombinant envelope assay (e.g., ENV 9). If a supplemental test is positive, the HIV-1 EIA, WB, and supplemental test should be repeated in 3 and 6 months. IVDU = intravenous drug user.

uals in each group will vary according to the time between initial and repeat HIV-1 testing, HIV risk status of the population tested, and the use of different commercial sources of EIA and Western blot kits at repeat testing.

Repeat EIA and Western blot 1 month after the initial IWB will often detect the seroconverters, as was demonstrated in three of the four seroconverters in this sample and in all 18 seroconverters in the series by Wilbur et al. [14]. If the EIA is persistently reactive and the Western blot becomes positive, but infection seems implausible based on the individual's risk history, an EIA and Western blot should be repeated on a subsequent sample. Among those individuals with persistently reactive EIAs and IWBs who have not seroconverted upon repeat testing 1 month later, the risk of seroconversion is probably low. Nonetheless, we recommend that high-risk individuals be followed for at least 6 months after their last potential exposure to HIV-1, or longer if they still engage in high-risk behavior, with repeat EIAs and Western blots at 3- to 6-month intervals. Horsburgh et al. [45] have reported that 50% and 95% of individuals will seroconvert within 3 and 6 months after acquiring infection, respectively.

Low-risk individuals with persistent IWBs with p24 bands should be followed for at least 3 months in case they have denied existing risk behaviors, with EIA and Western blot repeated at 3 months. Although the sensitivity of supplemental tests in detecting the infrequent seroconversion in such individuals will be difficult to measure, negative supplemental tests may be useful in reassuring them, especially in situations such as pregnancy and applications for insurance and immigration. Low-risk individuals with bands on Western blot other than p24 antibodies or Western blots that are negative on repeat testing can be reassured that they are not infected and advised that they do not need further serologic follow-up.

High-risk individuals who revert to a negative Western

blot or have bands other than p24 or envelope bands should be followed for 6 months after their last high-risk behavior to exclude seroconversion. Negative supplemental tests may allow cautious reassurance, although again the sensitivity of such tests in this setting is uncertain. The eventual utility of supplemental tests for help in managing such persons with IWBs will be determined by further information from clinical epidemiologic studies that assess the sensitivity and predictive value of supplemental tests. The reasons for false-positive supplemental test results, like those for IWBs, require further study.

Addendum

Since submission of this manuscript, two additional high-risk cases have seroconverted. Both had a p24 band on initial Western blot and seroconverted within 1 month of their initial IWB.

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